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INDIAN CHEMICAL SOCIETY

VOLUME XV

1938

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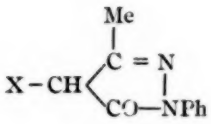
89	Formula (II)		
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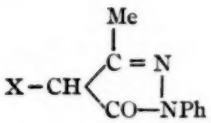
89	Formula (II)		
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THE PHYSICO-CHEMICAL PROPERTIES OF ASCORBIC ACID*

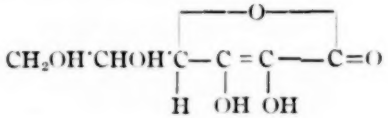
By Prof. J. C. Ghosh, D.Sc., F.N.I.

Fellows of the Indian Chemical Society, Ladies and Gentlemen,

I would, in the first place, take this opportunity of offering my best thanks to you for the honour which you conferred on me a year ago by electing me as your President. This is the highest gift which the chemists of India can offer to one of their Fellow-workers and I am very grateful to you all for your kind appreciation.

The Council expressed the wish sometime ago, that it was desirable that the old practice of the President addressing the Fellows at the annual general meeting should be revived ; I therefore make no apology in speaking to you on a subject in which I have been interested for some time.

Ascorbic acid, since its isolation in the pure state by Szent-Györgi and the elucidation of its constitution by the Birmingham School led by Haworth has been a material of such absorbing interest to all chemists that it was considered worth while to investigate its physico-chemical properties in detail. The constitution of ascorbic acid as given in (I) is now generally accepted as correct.



(I)

The hydroxy groups attached to the doubly linked carbon atoms have the remarkable property that they are both capable of easy oxidation and stepwise dissociation into H-ions. When ascorbic acid was first isolated, it was found that "it avidly took up oxygen" and legitimately suggested that by its power of reversible oxidation, it could function as an oxygen carrier. Kellie and Zilva[1] however proved that if ascorbic acid were dissolved in pure water distilled from quartz vessels, it was as stable as in the solid form. This observation was confirmed by Barron and co-workers[2] who found that recrystallised natural ascorbic acid

* The Presidential Address delivered at the fourteenth annual general meeting of the Indian Chemical Society held, in Calcutta on January 5, 1938.

free from the last traces of copper, if dissolved in the purest water, did not undergo any auto-oxidation up to p_H values 7·6. We have, however, found[3] that the behaviour of synthetic acid is different as regards its stability. Carefully purified synthetic ascorbic acid, when dissolved in purest water, gave results which did not agree with the observations of Zilva, Barron and co-workers. If auto-oxidation was estimated in a Warburg apparatus, oxidation between 70—90% took place in an hour within the p_H range 5·8 and 7·2. There are two possibilities—either our system contained traces of copper which is the most active catalyst known for this auto-oxidation or that pure ascorbic acid is capable of auto-oxidation but that the natural material used by Zilva and Barron contained traces of a very powerful protective agent.

Our reaction mixtures were accordingly tested for copper by rubeanic acid, and for copper and iron by sodium diethyldithiocarbamate with negative results. It might be contended that concentrations of copper, even lower than those detectable by these micro-reagents, could act catalytically. It was found that if copper salt were actually added to our solutions of ascorbic acid, so as to make its concentration of the order of 10^{-6} mols per litre, the auto-oxidation of ascorbic acid is not perceptibly accelerated at p_H 7. Further evidence that the auto-oxidation of ascorbic acid is not catalysed by copper when present in concentrations which could not be detected by rubeanic acid is obtained by the study of the action of potassium iodide on these systems.

The reaction

$$Cu^{++} + 2I^- \longrightarrow CuI + I$$

proceeds to completion towards the right if the iodine set free is used up to oxidise ascorbic acid present in excess. The solubility product of CuI is of the order of 10^{-12}; hence in a solution having a concentration of KI equal to 10^{-3} g. mol per litre, the concentration of Cu^{++} ion in presence of excess of ascorbic acid would be of the order of 10^{-9}. If copper were responsible for the auto-oxidation of ascorbic acid, KI would act as a retarding agent. KI in concentrations of the order of 10^{-3} g. mol per litre was found to have absolutely no action on the auto-oxidation of synthetic ascorbic acid which, therefore, is capable of spontaneous auto-oxidation. The conclusion becomes irresistible that the natural ascorbic acid used by Zilva, Barron (*et al*) contained traces of powerful protective agent. As to the exact nature of this protective system, significance attaches to the observation of Birch and Dann[4] that ascorbic acid and glutathione commonly occur in conjunction with one another.

They, in fact, described ascorbic acid and glutathione as linked factors in one system of oxidation in the animal cell. The reduction of dehydro-ascorbic acid by glutathione has been observed to be fairly rapid and is considered to be of considerable biological significance. Hence it was surmised that glutathione even in traces might protect ascorbic acid from auto-oxidation. Table I shows that sulphydril compounds, even in their oxidised form, can inhibit the auto-oxidation of synthetic ascorbic acid when present in order of concentrations 10^{-4} to $10^{-5}M$.

TABLE I.

Ascorbic acid = 0·004 g. = 260 c.mm. O_2. Soln. = 3 c.mm. Temp. = 30°.
Air = 48 c. mm.

Inhibitor.	Molar conc. of (A)	p_H.	C. mm. of O_2 absorbed in 60 min.	120 min.	180 min.	% of ascorbic acid left after 180 min.
	—	5·2	70	125	—	
Reduced glutathione	$1 \cdot 10^{-4}$	5·2	0	5	—	
	—	8·2	125	205	—	
	$1 \cdot 10^{-4}$	8·2	20	20	—	
	—	6·9	110	175	230	11
	$1 \cdot 10^{-3}$	6·9	20	45	50	81
	$1 \cdot 10^{-5}$	6·9	60	65	70	75
	$1 \cdot 10^{-6}$	6·9	120	155	190	27
Oxidised glutathione	$5 \cdot 10^{-4}$	6·9	20	40	45	85
	$2 \cdot 10^{-4}$	6·9	5	0	20	92
Cystein	$2 \cdot 10^{-3}$	6·9	0	10	15	97
	$2 \cdot 10^{-5}$	6·9	0	5	5	98
	$5 \cdot 10^{-6}$	6·9	0	0	5	98
	$2 \cdot 10^{-7}$	6·9	80	160	185	28
Cystin	$14 \cdot 10^{-4}$	6·9	25	25	30	88
	$14 \cdot 10^{-6}$	6·9	20	25	20	92
Sulphuretted hydrogen	$14 \cdot 10^{-5}$	6·9	0	0	10	96
	$14 \cdot 10^{-6}$	6·9	75	120	185	28

Similar results were obtained with thiolactic acid (*vide* Table IA).

TABLE Ia.

Soln. = 1·2 c. mm. Air = 16·8 c. mm. Ascorbic acid = $1 \cdot 10^{-2}$ *M*. Temp. = 28°.

p_H. (Phosphate).	Conc. of thiolactic acid.	C. mm. of oxygen absorbed in 30 min.	60 min.	90 min.	120 min.
5·2	—	16	—	34	37
5·2	$1 \cdot 10^{-5}$ mol.	17	—	34	37
5·2	$1 \cdot 10^{-4}$	0	0	0	0
6·0	—	24	36	49	60
6·0	$1 \cdot 10^{-5}$	12	16	19	24
6·0	$1 \cdot 10^{-4}$	0	0	0	0
7·0	—	61	86	103	—
7·0	$1 \cdot 10^{-5}$	64	82	—	—
7·0	$1 \cdot 10^{-4}$	0	0	0	—
8·0	—	—	91	108	—
8·0	$1 \cdot 10^{-5}$	64	96	110	—
8·0	$1 \cdot 10^{-4}$	0	0	0	—

The action of mercuric chloride on these systems yields very interesting results. When added to ascorbic acid solutions at p_H 7, it has no effect whatever on the velocity of auto-oxidation. $HgCl_2$, however, easily combines with sulphydril compounds giving products which are extremely insolubie ; hence it is to be expected that the protective action of sulphydril compounds may be removed by the action of $HgCl_2$. Table II shows that this is actually the case.

TABLE II.

Ascorbic acid = $1 \cdot 10^{-2}$ mol. Temperature = 20°. p_H = 7·0.

Conc. of $HgCl_2$.	Conc. of -SH.		C. mm. of oxygen absorbed in 30 min.	60 min.	90 min.
—	—		61	85	103
—	$5 \cdot 10^{-6}$ mol.	(Cystein)	0	0	0
—	$1 \cdot 10^{-5}$	(Glutathione)	0	0	0
$1 \cdot 10^{-4}$ mol.	—		60	84	107
—	$5 \cdot 10^{-6}$	(Cystein)	65	80	102
—	$1 \cdot 10^{-5}$	(Glutathione)	32	46	—

It appears, therefore, that glutathione and cystein are inhibitors and copper is an accelerator of the spontaneous process of auto-oxidation of ascorbic acid. The interaction of these two factors will be clear from Table III.

TABLE III.

Cystein and Copper.

Ascorbic acid = 0·004 g. Temp. = 30°. Soln. = 3 c. mm. Air = 48 c. mm.

Amount of Cystein—HCl.	Amount of $CuSO_4$, $5H_2O$.	Cu : cystein.	C. mm. of oxygen absorbed in 60 min.	120 min.	180 min.
—	—	—	120	185	220
—	$75 \cdot 10^{-6}$	—	140	165	180
$1 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	1 : 1 mol.	0	0	0
$1 \cdot 10^{-5}$	$37 \cdot 10^{-5}$	5 : 2	—	180	225

The inhibition is complete when the ratio of copper to sulphydril compound is 1:1 and the protective action of sulphydril compound is removed when its ratio with copper is as 2:5. Besides the specific protective action of sulphydril compounds present in minute traces, it has been observed that various amino-acids can inhibit the oxidation of ascorbic acid when present in equivalent proportions.

Ascorbic acid is also found widely distributed in the plant kingdom. Giroud[5] has found that the ascorbic acid content of plant leaves and tissues varies with their chlorophyll contents, is absent from plant grown in darkness and diminishes in leaves in autumn when chlorophyll disappears. Accordingly the inhibitory effect of chlorophyll on the auto-oxidation of ascorbic acid was studied. The purest samples of chlorophyll, α and β, were obtained from Prof. Stoll, and the results are indicated in Table IV.

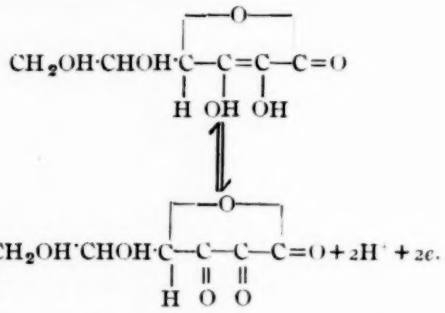
TABLE IV.

Conc. of ascorbic acid = 1×10^{-2}. p_H = 7·0. Temp. = 25°.

Conc. of chlorophyll.	C. mm. of oxygen absorbed after 0 min.	60 min.	120 min.	240 min.
Chlorophyll-α.				
Nil	0	65	86	92
2×10^{-4}	0	0	0	0
2×10^{-5}	0	45	65	68
Chlorophyll-β.				
Nil	0	65	86	92
2×10^{-3}	0	0	0	0
2×10^{-4}	0	0	0	0
2×10^{-5}	0	69	78	84

It is clear that chlorophyll at concentrations of the order of $10^{-4.5}$ completely protects ascorbic acid from oxidation by oxygen. It thus appears that wherever ascorbic acid is found in the plant and animal kingdom, nature always associates it with materials which prevent its oxidative destruction.

It has already been noted that if ascorbic acid were to function as a carrier of oxygen in the cell, it should be capable of reversible oxidation and reduction according to the equation



The thermodynamic equation for the electromotive force of such a system should be

$$E = E_0 - \frac{RT}{F} \cdot p_H + \frac{RT}{2F} \log \frac{[Ox]}{[Red]}.$$

If the oxidant and reductant undergo ionisation into H^+ and an anion, the equation has to be modified thus:

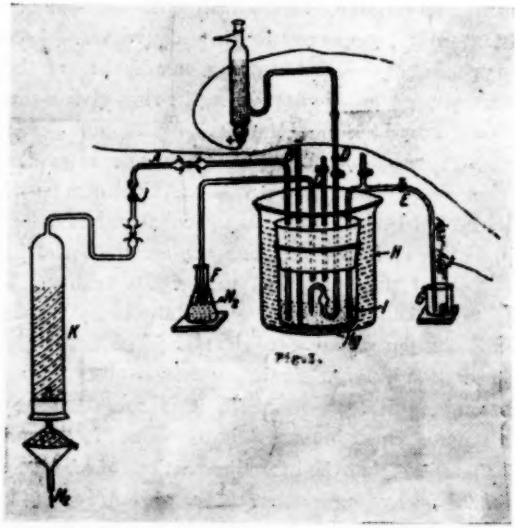
$$E = E_0 - \frac{RT}{F} \cdot p^H + \frac{RT}{2F} \cdot \log \frac{[Ox]}{[Red]} + \frac{RT}{2F} \log \frac{1 + \frac{[H^+]}{K_0}}{1 + \frac{[H^+]}{K_r}}$$

where K_0 is the dissociation constant of the oxidant and K_r, the dissociation constant of the reductant. Early workers reported extremely discordant results. Thus Green[6], Karrer[7], Laki [8] reported values of potentials given by the reductant alone, which are, therefore, thermodynamically meaningless. The difficulties that have been encountered in measuring the reversible potential of this system are due to the fact that the system is electromotively sluggish and that it requires complete elimination of last traces of oxygen from the electrode surface and the solution. Borsook and co-workers[9, 10] with the aid of special vacuum technique have been able to obtain reproducible values of reversible potentials. The difficulties due to instability of

dehydroascorbic acid have been unnecessarily exaggerated. We have found for instance that if ascorbic acid is oxidised by bromine water, and the resulting solution kept in buffer mixtures, whose p_H did not exceed 7·5 for 3 hours in contact with air, it is possible to recover the whole of the ascorbic acid by treatment with sulphuretted hydrogen

We have applied to this system the technique of measuring thermodynamic redox potentials which were developed in connection with our studies of the potential of sulphydril bodies.[16, 17] The essence of the process consists in subjecting the solution of the oxidant to cathodic reduction on the surfrce of an electrode for some time thereby removing simultaneously the last traces of oxygen from the surface of the electrode and the bulk of the solution, and then measuring the steady potential attained by this electrode when pure nitrogen has been passed through the solution for several hours. The experimental arrangement is shown in Fig. 1.

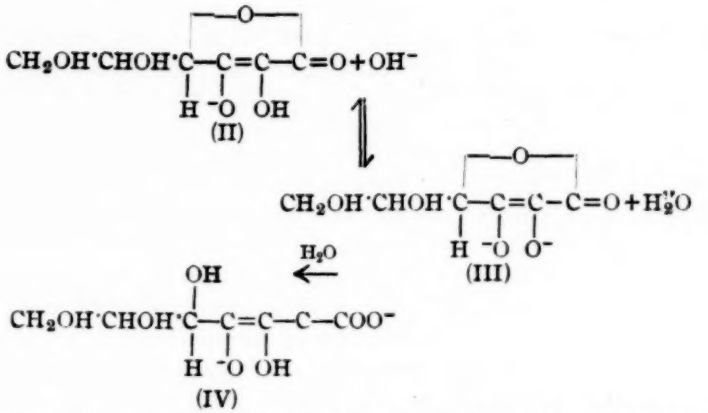
FIG. 1.



A – Nitrogen inlet; B–Connection with the cathode; C—For withdrawal of solution from the cell for analysis; D—Decinormal calomel electrode; E—Siphon tube for connection between anode and cathode; F—Nitrogen outlet dipping in a water-trap; G–Anode chamber; H –Water-bath at constant temperature; I—Reaction cell-cathode chamber; J—Cotton-plug bulbs; K—Alkaline pyrogallol; L—Jena gas filter.

Buffered solutions of ascorbic acid were partially oxidised by means of bromine, and introduced into the cathode chamber (I) and the anode chamber (G), which were connected together by the agar bridge (E). A current of 10 milliampers for an hour was used for complete removal of oxygen and partial reduction of dehydroascorbic acid. A current of nitrogen was then passed through the cathode chamber for 6 hours, and the steady potential of the electrode in this chamber was measured against the decinormal calomel electrode (D). The ratio of ascorbic to dehydroascorbic acid in the solution was measured by pipetting out a part of the solution through (C) and analysing it iodometrically. E_0 has the value $-0{\cdot}384$ at 30°and the thermodynamic equation is valid within the p_H range 2·5 and 7·5. Ball [11] has just reported a change in the slope of the E. M. F and p_H curve in the neighbourhood of p_H 5. We have not observed this anomaly. Nor is this anomaly to be expected if the dissociation constants of ascorbic acid and dehydroascorbic acid are of the same order of magnitude, as has been actually found to be the case.

The molecular conductivity of pure ascorbic acid [12] has been determined at various dilutions and the first dissociation constant has been found to be $6{\cdot}3 \times 10^{-5}$. Birch and Harris [13] have also obtained similar values of dissociation constant from potentiometric titration curves. Ascorbic acid does not undergo opening of the lactone ring on addition of the first equivalent of alkali. One of the enolic hydroxyls in the ring system must, therefore, be responsible for the production of H^+ ions. Feiser and Feiser [14] have shown that 2-hydroxy-1:4-naphthoquinone, which has got a similar ring structure, gives a value of dissociation constant almost identical with that of ascorbic acid. Studies by Reichstein, Haworth and collaborators [15, 15a] on the action of limited quantities of diazomethane on ascorbic acid have shown that the enolic group at position 3 has the greater acidity. The addition of one equivalent of alkali to ascorbic acid (rotation + 24°) gives a product (II) which has a molecular rotation of + 115°. The first ionisation, therefore, increases the rotation by 91°. If the rotation is observed immediately after adding a second equivalent of alkali, it is fouud to be + 200° approximately. This almost equal increment in the value of molecular rotation lends support to the view that the immediate action of the second equivalent of alkali is to ionise the hydrogen atom of the second hydroxyl group without opening the lactone ring. This form (III) having molecular rotation of + 200° is extremely unstable and tautomerises with the opening of the lactone ring into a form (IV) which has a molecular rotation of + 130°. It may be noted that on addition of two equivalents of HCl the original ascorbic acid is recovered. The equilibrium between the lactone and open-chain form in presence of OH^- according to equation,



admits of quantitative estimation by the use of experimental data obtained from potentiometric titrations in the region of p_H between 10 and 11. This has been done and the value of the equilibrium constant is

$$K_{2L} = \frac{[OH^-][\text{Monovalent lactone anion}]}{[\text{Divalent open-chain anion}]} = 2{\cdot}5 \times 10^{-3} \text{ at } 27°.$$

The actual experimental data is given in the Table V.

TABLE V.

0·2*M*-NaOH soln. Measured against normal calomel electrode at 27°. The system was thoroughly saturated with H_2. 50 C.c. of *M*/100-ascorbic acid titrated.

Alkali added.	E.M.F.	p_H.	$K \times 10^{-3}$.
2·68 c. c.	0·895	10·200	2·74
2·76	0·905	10·359	2·40
2·88	0·915	10·533	2·51*
3·03	0·923	10·667	2·45
3·51	0·942	10·983	2·59

Mean $K = 2{\cdot}5 \times 10^{-3}$

N. B. * A typical calculation may be shown as follows. Taking the figures marked with an asterisk in the above table, we have, the free alkali remaining in the system at equilibrium calculated from p_H, 10·533 = 0·00034*M*.

The amount of alkali added = 2·88 c. c. of which 2·5 c. c. being used up by the formation of monosodium salt, hence alkali really available for second step dissociation = 0·38 c. c. in 52·88 c. c solution.

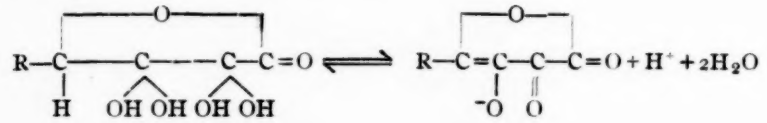
Conc. of alkali added = 0·0015*M*.

Conc. of divalent open-chain anion = 0·0015 − 0·00034*M*.

Conc. of monovalent lactone anion = {0·01 − (0·0015 − 0·00034)}*M*.

$$K = \frac{(0{\cdot}00034) \times \{0{\cdot}01 - (0{\cdot}0015 - 0{\cdot}00034)\}}{(0{\cdot}0015 - 0{\cdot}00034)} = 2{\cdot}51 \times 10^{-3},$$

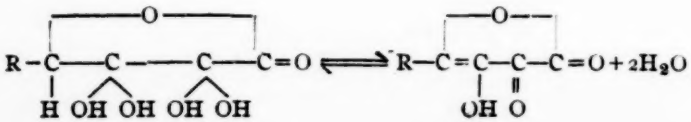
It is necessary to discuss the physico-chemical properties of dehydroascorbic acid at some length as our results are at considerable variance from those of Hirst, Herbert (*et al*).[18] They find that the oxidation product of ascorbic acid, newly formed by the action of I_2, has no selective absorption band characteristic of ascorbic acid and is neutral in reaction. The latter observation is based on very doubtful evidence. We have been able to prepare solutions of dehydroascorbic acid free from association with any other electrolyte by the action of hydrogen peroxide in presence of colloidal platinum. The solution has a molecular conductivity of 33·8 at $v=100$, $t=23°$. It has an acid reaction, almost identical with that of ascorbic acid, and its dissociation constant as determined from conductivity data is $12·6\times 10^{-5}$. The absence of selective absorption band in the ultraviolet has led to the adoption of formula (V) for the newly formed dehydroascorbic acid which has a molecular rotation of $+56°$



(V) (VI)

Perhaps this form dissociates after dehydration into anion (VI) and H^+.

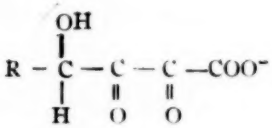
Addition of one equivalent of alkali to dehydroascorbic acid leads also to the production of anion (VI) with a molecular rotation of $-26°$. The proof that the lactone ring does not open at this stage is supplied by the fact that it is possible to recover ascorbic acid in 95% yield from this solution by treatment with H_2S. If to this salt solution an equivalent of mineral acid is added, a product is obtained whose molecular rotation is $-10°$ which is practically the same as that obtained by Herbert (*et al*), for a solution of dehydroascorbic acid three days after preparation. This old sample develops a selective absorption band in the ultraviolet almost similar to that of ascorbic acid. It appears, therefore, very probable that in an old sample of dehydroascorbic acid equilibrium exists between forms (V) and (VII) thus



(V) (VII)

The conclusion of Hirst (*et al*), that the neutral salt is formed from dehydroascorbic acid by the opening of the lactone ring is untenable, for in that case ascorbic acid could not have been recovered by simple treatment with H_2S. On addition of excess of alkali, however, the lactone ring opens up and an open-chain anion is formed.

It has been generally accepted that dehydroascorbic acid is a monobasic acid, and that the open-chain anion has the constitution (VIII)



(VIII)

Potentiometric titrations with a strong alkali in a rapid current of hydrogen, which prevents further oxidation and rupture of the molecule, lead to different conclusions. Concordant equilibrium constants are obtained if we assume a reversible transformation of the type,

$$\text{Anion of lactone} + OH^- \rightleftharpoons \text{open-chain divalent anion.}$$

The equilibrium constant has the value $3{\cdot}8 \times 10^{-5}$ (approx.).

As in the case of the second step ionisation of ascorbic acid, the opening of this lactone into an open-chain divalent anion was followed from the potentiometric titration of dehydroascorbic acid. The results are given in Table VI.

TABLE VI.

Alkali (NaOH) = 5/18 *N*. *M*/110-Dehydroascorbic acid (50 c.c.), measured against *N*/10-calomel electrode at 21°, saturated with H_2 gas.

Alkali added.	E. M. F.	p_H	$K \times 10^{-5}$.
2·60	0·920	9·75	4·40
3·20	0·961	10·43	3·51
3·40	0·971	10·60	3·62
			Mean $3{\cdot}6 \times 10^{-5}$

This open-chain anion can only give back ascorbic acid by treatment with concentrated HI. In alkaline media, it is a powerful reducing agent, being further oxidised in the cold by $AgNO_3$, and H_2O_2 with colloidal Pt. It is very unstable and decomposes easily in presence of air into oxalic acid and *l*-threonic acid.

It is well known that *d*-ascorbic acid does not possess the biochemical properties of its optical isomer, the vitamin C. It was, therefore, worthwhile investigating more fully the anisotropic properties of the molecule. Ascorbic acid has an absorption band in the ultraviolet, the band head being at about 265 $\mu\mu$. We have measured the circular dichroism, *i.e.*, the difference in the extinction coefficients for *d*- and *l*-circularly polarised light in this region by a method which has already been described in a recent issue of our Journal (1937, **14**, 495).

A large difference has been observed and the results are given in Table VII.

TABLE VII$_a$

Wave-length = 2536 $\mu\mu$.

Conc. of ascorbic acid.	$g = \frac{\epsilon_l - \epsilon_r}{\epsilon_a}$.
0·0004*M*	0·0428
0·00015*M*	0·0373
	Mean 0·0400

The sodium salt of dehydroascorbic acid gives a pale yellow solution, which shows circular dichroism even in the greenish blue region. A monochromatic plane polarised beam of light on passing through such a solution, becomes elliptically polarised due to differential absorption of the two oppositely circularly polarised components. The experimental results are given in Table VIII.

It is clear that several hydrogen atoms in the molecule are extremely labile. They are responsible for easy dehydrogenation and the numerous stepwise ionisation of the molecule These changes on closer examination appear to be of a protein character and much patient investigation will be necessary before the nature of these complex transformations are completely cleared up.

TABLE VIII.

Measurement of rotatory dispersion and ellipticity of dehydroascorbic acid as determined by Bruhat's apparatus (*Bull. Soc. chim.*, 1930, *iv*, **47**, 251) in the visible region.

Conc. of dehydroascorbic acid (to which had been added 3 equivalents of caustic soda) = $M/100$.

Wave-length.	Length of the soln.	Rotation.	Sp. rotation.	Ellipticity.	$\epsilon_l - \epsilon_r$ calc. from eq.*	Mol. extin. coeff. ϵ.	g (Anisotropic factor).
6438 Å	20 cm.	−0°06	−17°	...	...	...	...
6200	,,	−0°06	−17°	...	...	...	...
6000	,,	−0°07	−20°	...	...	...	...
5780	,,	−0·07	−20°	...	...	...	...
5460	,,	−0°08	−22°	...	...	...	...
5200	5 cm.	−0°03	−34°	...	...	...	...
4938	2 cm.	−0°01 ?	...	−0°03 (right handed)	0°045	10°7	0°0042
4738	,,	−0°01 ?	...	−0°04 (,,)	0°06	15	0°004
4538	1 cm.	?	...	−0°02 (,,)	0°06	23°7	0°0025

* Ellipticity = ϕ (in radians) = $\frac{[\epsilon_l \times \epsilon_r].\ c \times l}{4 \times 0{\cdot}4343}$; c = Molar conc. ; l = length in cm.

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VITAMIN C CONTENTS OF INDIAN FOODSTUFFS. CHILLIES (*CAPSICUM*).

By C. A. Rothenheim, H. S. Shaik Mahamud and S. S. Cowlagi.

The ascorbic acid contents of several varieties of chillies (*Capsicum*) found around the city of Bombay have been determined. A modified method of Tillmans using 2 : 6-dichlorophenolindophenol has been used. The reversibly oxidised ascorbic acid has been reduced by hydrogen sulphide. The values of ascorbic acid thus obtained are considerably higher. The ascorbic acid content of ripe chillies is greater than that of green ones. Chillies do not contain iron.

Though a large amount of work has been done on the vitamin content of foodstuffs, there is still a wide field for the standardisation of the vitamin contents of Indian plants and foodstuffs, especially in the different provinces where the same material contains varying amounts of the vitamins. It is also observed that different varieties of the same plant contain widely different amounts of the vitamin. This paper deals with the ascorbic acid content of chillies which are found round about the city of Bombay. The chillies for these experiments were obtained through the Superintendent of the Crawford market who had kindly arranged to supply us with the freshest material available in Bombay.

Experimental.

In estimating ascorbic acid, the micro-chemical method developed by Tillmans *et al* (*Z. untersuch. Lebensmit*, 1932, **63**, 1, 21, 276) as modified by Harris and Ray (*Biochem. J.*, 1933, **27**, 590) and Birch, Harris and Ray (*Biochem. J.*, 1933, **27**, 303) was followed. The above method of titration with 2:6-dichlorophenolindophenol does not hold good if iron is present in the extract of the vegetable matter. It is also known that phenols, cysteine, yeast extracts and tannins are interfering substances. It is further known that certain naturally occurring reducing agents such as glutathione and certain phenolic compounds tend to reduce the indicator in neutral or alkaline solutions. The titrations were, therefore, carried out in acid solution and the chillies were carefully tested for the presence of iron and phenolic bodies. For this purpose about 10 g. of chillies were cut into small pieces and were boiled in a dish with about 75 ml. of 3% hydrochloric acid. The boiling was continued till about two-thirds of the liquid

evaporated. This was then filtered and the filtrate tested for iron. No trace of iron was detected in any of the varieties of chillies examined. A portion of the above filtrate was used to detect the presence of phenolic and reducing bodies and none of the varieties gave the characteristic colour with ferric chloride or any precipitate with mercuric acetate.

In estimating ascorbic acid by Tillmans method we used a well known make of ascorbic acid as a standard.

The ascorbic acid solution was made by dissolving pure ascorbic acid in redistilled water. The solution was stored in the dark in the refrigerator in an atmosphere of carbon dioxide. Under these conditions it was found to be fairly stable but daily standardisation was necessary as shown in Table I.

TABLE I.

Date.	Ascorbic acid content in 10 ml. soln.
9-6-1937	0·8676 mg.
15-6-1937	0·7934
22-6-1937	0·7807
1-7-1937	0·7778

Though there is seen a marked fall in the ascorbic acid content of the solution over a period of seven days, it was found to be constant during the course of a day. For this reason, it is desirable to standardise the solution before any estimation of the vegetable extract is undertaken, though for subsequent titration during the day the strength of the solution is constant.

The standardisation was carried out in presence of dilute sulphuric acid against 0·01*N*-iodine solution using freshly prepared starch solution as indicator. The concentration of ascorbic acid was so adjusted that 1 ml. of 0·01*N*-iodine was required for 10 ml. of ascorbic acid solution. Each ml. of 0·01*N*-iodine is equivalent to 0·88 mg. of ascorbic acid.

The indicator solution was made by dissolving 0·1 g. of 2:6-dichlorophenolindophenol in 50 ml. of hot phosphate buffer solution of p_H 7·2. When prepared in this way it is fairly stable, but like ascorbic acid this solution requires daily standardisation. Table II gives the range of the fall of the concentration of the indicator solution expressed in equivalents of ascorbic acid.

TABLE II.

Date.	Ascorbic acid equivalent of 1 ml. of the indicator solution.
9-6-1937	0·9947 mg.
15-6	0·9028
22-6	0·8685
1-7	0·7778

Thus a daily titration of the indicator solution with a standardised solution of the ascorbic acid solution gives the best results in the estimation of the extract. In standardising the indicator, it is necessary to preserve the same general conditions regarding acidity and dilution, which are present, while titrating the unknown ascorbic acid content of the vegetable extract. In the present case 0·05 ml. of the indicator solution from a 1 ml. microburette was run into a small conical flask to which were previously added 1 ml. of distilled water and 1 ml. of glacial acetic acid. This was then titrated against the ascorbic acid from a microburette till the red colour which the indicator immediately assumes in acid solution is just discharged. Special care was taken to see that the flask is constantly shaken during the titration and the whole operation is finished within 1-2 minutes.

Extraction.—About 30 g. of chillies were weighed after removing the stalks. The chillies were then ground on an Indian grinding stone. As the surfaces of the grinding stones are rough no sand is necessary and the material is obtained in a very finely divided pasty mass. During the process of grinding 5 ml. of 20 % trichloroacetic acid, 20 ml. of distilled water and 1 ml. of 0·1*M*-potassium cyanide were added gradually. Potassium cyanide was added following the observation of McHenry and Graham that it has the stabilising effect on the ascorbic acid present in the material. The finely ground pulp so obtained was centrifuged for 5-7 minutes at 3000 r. p. m. The supernatant liquid was decanted and the residue similarly extracted twice in the centrifuge cups with 17 ml. of 3% trichloroacetic acid and 5 drops of 0·1*M*-potassium cyanide. The volume of the combined supernatant liquid was measured and the liquid was filtered through a Buchner funnel.

The filtrate was then titrated from a microburette against 0·1 ml. of standard indicator solution to which 2 ml. of water and 2 ml. of glacial acetic acid were added following the observation of Ghosh and Guha (*J. Indian Chem. Soc.*, 1935, **12**, 30) that the addition of acetic acid inhibits the spontaneous decolouration of the dye in the presence of trichloroacetic acid;

The indicator was diluted with water, following the observation of McHenry and Graham (*Biochem. J.*, 1935, **29**, 2013) that the time required for the given amount of trichloroacetic acid to decolourise a fixed amount of indophenol solution is considerably increased when the indophenol is diluted with 15-20 volumes of water and at the same dilution the time required for the reduction of the dye by ascorbic acid is only slightly prolonged. As in the previous case the titrations were carried out within 1-2 minutes. We have also observed, as did Ghosh and Guha, that a very faint pink colour persists at the end of the titration but the error from this source may be avoided by comparing the titration flask with a control. Table III gives typical results of a number of analyses of various chillies examined by us.

TABLE III.

Name of the varieties.	Condition of the chillies.	Ascorbic acid in mg. per g.	
		By direct extraction.	By reduction of extract with H_2S.
Jadi mirchi (Panvel.) *Capsicum grossum*	4 days old	0·042	0·230
,,	Fresh	0·061	0·431
,,	,,	0·168	0·495
,,	,,	0·095	0·572
,,	Ripe	0·115	0·661
Long, light green variety	Fresh	0·046	0·303
,,	,,	0·047	0·328
Long, dark green shining variety	,,	0·066	0·331
Lavangi, very small variety, *Capsicum minimum*	Ripe	0·101	0·248

In all our experiments green chillies were used in as fresh a condition as they could be available. Where ripe chilly is indicated they are red in colour and fully mature while others are green. The above values represent the more typical results from a number of analyses. From Table III it will be seen that the ascorbic acid content varies greatly with different varieties

of chillies. It is also seen that there is appreciable difference in different samples of the same variety. Among the varieties examined by us, the jadi mirchi or *Capsicum grossum* from Panvel gives higher vitamin content on the average. It is interesting to note that the ripe chillies contain a proportionately higher amount of the ascorbic acid. This is especially clear from Lavangi or *Capsicum minimum* in which case both the green and the ripe (red) samples were plucked at the same time from the tree and examined for ascorbic acid with the minimum delay. It seems that the formation of ascorbic acid attains the highest level in the ripe chillies.

Reduced and Reversibly Oxidised Ascorbic Acid.

Tillmans, Hirsch and Jackish observed that the titration value of cucumber extracts was considerably increased when the extracts were kept in an atmosphere of hydrogen sulphide for several hours. Similar results were obtained by McHenry and Graham in case of onions and carrots. In our experiments, the method of McHenry and Graham was followed. The chilly extract was diluted with an equal volume of water so that the concentration of trichloroacetic acid was 1·5 per cent. Hydrogen sulphide was then bubbled in for 10 minutes. The flask was tightly corked and left in the refrigerator overnight. Hydrogen sulphide was removed on the following day by passing a stream of carbon dioxide for two hours till there was no trace of sulphide. The ascorbic acid content was then estimated as previously stated. The results of this reduction of the partially oxidised ascorbic acid are given in the last column of Table III.

It will be seen that the value of the ascorbic acid after reduction is very considerable. There is also a rough proportionality between the free ascorbic acid and the ascorbic acid after reduction, in an inverse proportion. Thus where the free acid is low in concentration the reduced acid is considerably higher. It is thus interesting to note that the ascorbic acid always exists in the plant material as a reversibly oxidised derivative along with the free acid.

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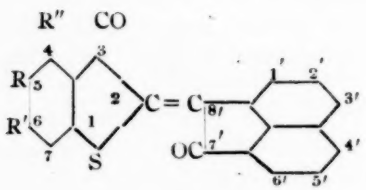
DYES DERIVED FROM ACENAPHTHENEQUINONE. PART VI. 2-(4-METHYL)-THIONAPHTHENEACENAPHTHYLENE-INDIGOS.

By Sisir Kumar Guha.

A few vat dyes have been prepared by the condensation of 4-methyl-3-hydroxythionaphthene with acenaphthenequinone and its derivatives. The dyeing shades developed on cotton and on wool from these compounds have been compared with those obtained from 2-thionaphthene-acenaphthylene-indigos and its 5-methy land 6-methyl derivatives The effect of methyl group in the 4-position of the thionaphthene nucleus of the molecule of the parent compound is to lighten the colour. It has also been found that as far as the colour is concerned the 4-methyl compounds occupy an intermediate position between the 5-methyl and the 6-methyl derivatives, and the 5-methyl dyes possess the deepest shade. The microphotographs of the spectra of the dyes of the three series have been taken and the absorption maxima determined.

Of the four theoretically possible isomeric methyl-3-hydroxythionaphthenes, the 5-methyl and the 6-methyl derivatives (Auwers and Arndt, *Ber.*, 1909, **42**, 541 ; Friedlander, **9**, 589 ; Auwers and Thies, *Ber.*, 1920, **53**, 2285) were condensed with various acenaphthenequinones respectively and some valuable vat dyes were obtained of the general formula (I, R = Me, R′ = R″ = H ; R′ = Me, R = R″ = H) (Guha, *J. Indian Chem. Soc.*, 1933, **10**, 682 ; 1936, **13**, 94). It was observed that Martinet's rule (*Rev. Gen. Mat. Col.*, 1921, **25**, 17) holds good in the acenaphthenequinone series also as far as the substitution of one Me group in the thionaphthene nucleus of the molecule of 2-thionaphtheneacenaphthyleneindigo is concerned.



(I)

In the present paper methylthionaphthene-acenaphthyleneindigos are

described of the general formula (I, R″=Me ; R=R′=H) obtained by condensing 4-methyl-3-hydroxythionaphthene (*Chem. Zentrl.*, 1928, **99**, I, 759 ; E. P. 279489 ; U. S. P. 28037) with acenaphthenequinone and its various derivatives.

7-Methyl-3-hydroxythionaphthene has not been isolated and not described in literature although 7 : 7′-dimethylthioindigo is mentioned in D. P. 241910 (*cf.* Thorpe and Ingold, " Vat Colours", 1923, p. 129). So the preparation of the dyestuffs described here has enabled the author to make a complete study, up till now, of the influence of one Me substitution upon the colour of the parent dye, commercially known as Ciba Scarlet G which is so particularly important for printing, and its various derivatives when the methyl substitution occurs in the 4, 5 or 6 position of the thionaphthene nucleus of the molecule (E. P. 344/08 ; U. S. P. 891690; G. P. 205377 ; Bezdzik and Friedlander, *Monatsh.*, 1908, **29**, 386 ; Staudinger, Goldstein and Schlenker, *Helv. Chim. Acta*, 1921. **4**, 342 ; E. P. 20003/08 ; G. P. 213504 ; Mayer and Schönfelder, *Ber.*, 1922, **55**, 2972 ; Guha, *J. Indian Chem. Soc.*, 1932, **9**, 423).

It has been observed from a comparison of the dyeing shades on wool and on cotton obtained from 2-thionaphtheneacenaphthyleneindigo and its various methyl derivatives that the entrance of Me-group into the 6-position, *i.e.*, in the *para* position to carbonyl group of the parent dye, lightens the colour considerably and this "hypsochromic" effect is very prominent. Again the entrance of the same group in the 5-position *i.e.*, the *meta* position to CO-group of the parent dye has a marked deepening effect. The dyestuffs described here show that when the Me-group is introduced in the 4-position, *i.e.*, in the *ortho*-position to CO-group, the colour is again lightened, but these 4-methyl compounds are deeper in colour than those of the corresponding 6-methyl compounds in this series. So it is concluded that the lightening of colour of isomeric thioindigoid dyes is most marked when the Me-group is introduced into the 6-position and the deepening effect is quite prominent when it is introduced in the 5-position of the thionaphthene nucleus of the molecule as discussed in Martinet's rule.

The colours of the dyeings on wool and on cotton of the various methylthionaphtheneacenaphthyleneindigos are given in Table I.

The absorption spectra of the substance of the three series, mentioned in Table I, have now been taken, the examination of which (Fig. 1, 2, 3 and 4) points out that the heads of the absorption bands in case of 5-methyl compounds are shifted much towards the red end of the spectrum ; then

come the heads of the bands of the 4-methyl compounds and last are those of the 6-methyl compounds. This result is in quite harmony with what was realised qualitatively and described before. All the solutions examined were in xylene and the absorption spectra of these dyes have been considered in the visible part of the spectrum as the colours of the dyestuffs have to be judged only. The absorption spectra were taken by Messrs Adam Hilger's constant deviation spectrograph. The source of light was a straight filament and argon comparison was made use of. The microphotographs of the spectra were taken with the Zeiss microphotometer and the positions of the argon lines were marked.

These dyes like the isomeric 5-methyl and 6-methyl varieties were obtained easily in crystalline form. They develop lustre when rubbed, volatilise when heated strongly above their melting points. The mother compound, its chloro and bromo derivatives, when treated with strong sulphuric acid, produce deep bluish green solutions and the methoxy derivative gives rise to blue solution from which on the addition of water, the dyes are reprecipitated unchanged suitable for dyeing on wool from an acid bath. The colours of the vat obtained from them by alkaline hydrosulphite resemble those of the corresponding 6-methyl compounds. The dyeing shades on wool and on cotton have been fully developed in all the cases except the methoxy derivative, which in case of cotton only behaved towards the alkaline reducing agent in the same way as the isomeric dyes. The faint violet-red vat obtained with great difficulty in this case turned into faint pink by atmospheric oxidation. The dyes are all soluble in pyridine, nitrobenzene, xylene and chloroform.

It is interesting to note (*cf.* Table I) that in the case of the mother compound, its chloro- and bromo derivatives there is a systematic gradual rise of melting point as the Me-group in the thionaphthene nucleus in the molecule of 2-thionaphtheneacenaphthyleneindigo is shifted from 4 to 5 and then to 6-position. But in the case of methoxy derivatives there is fall of m. p. in the 5-methyl compound and again rise in case of the 6-methyl derivative.

The 4-methyl-3-hydroxythionaphthene, utilised in this investigation, has been described in various Patents (*loc. cit.*) but nothing is recorded regarding appearance, melting point and solubility, which together with other details, have been mentioned in the experimental part.

TABLE I.

A = Acenaphthylene indigo.

Names of the compounds.	M..p.	Shades.		Absorption maxima.
		on wool.	on cotton.	
2-(4-Methyl)-thionaphthene-A	263°	Vermillion	Vermillion	4735 Å
2-(5-Methyl)-thionaphthene-A	265°-66°	Scarlet-red	Scarlet red	4985
2-(6-Methyl)-thionaphthene-A	305°	Vermillion	Vermillion	4622
2-(4-Methyl)-thionaphthene-8′-(3′-chloro)-A	270°-71°	Red	Red	4750
2-(5-Methyl)-thionaphthene-8′-(3′-chloro)-A	284°-85°	Bright scarlet red	Deep scarlet red	4987
2-(6-Methyl)-thionaphthene-8′-(3′-chloro)-A	297°	Vermillion	Vermillion	4655
2-(4-Methyl) thionaphthene-8′-(3′-bromo)-A	260°	Red	Red	4800
2-(5-Methyl)-thionaphthene-8′-(3′-bromo)-A	282°	Deep scarlet red	Deep scarlet red	4992
2-(6-Methyl)-thionaphthene-8′-(3′-bromo)-A	302°	Bright vermillion	Light red	4703
2-(4-Methyl)-thionaphthene-8′-(1′-methoxy)-A	Above 305°	Deep red	Faint pink	4810
2-(5-Methyl)-thionaphthene-8′-(1′-methoxy)-A	279°-80°	Deep red	Pink	4995
2-(6-Methyl)-thionaphthene-8′-(1′-methoxy)-A	300°	Deep red	Faint pink	4783

EXPERIMENTAL.

4-Methyl-3-hydroxythionaphthene.—The diazotised solution of (5 g. of) aminotolunitrile (NH_2 : CN : Me = 1:2:3) (Gabriel and Thieme, *Ber.*, 1919, **52**, 1082; Kenner and Witham, *J. Chem. Soc.*, 1921, **119**, 1458) was poured into a cooled aqueous solution of potassium xanthogenate (7 g.) containing anhydrous sodium carbonate (14 g.) and the solution stirred; the sulphur-yellow crystalline precipitate, which first appeared was quickly transformed into a brown tarry mass with evolution of nitrogen. When the brown product became solid after standing, it was dissolved in hot sodium hydroxide (35%) and a little water; the resulting solution was heated at 100° on the water-bath with sodium-monochloroacetate (5 g.). By acidification 3-methylbenzene-1-thioglycollic-2-carboxylic acid nitrile was obtained. It

was collected and as indicated in E. P. (*loc. cit.*) it was converted into the blue crystalline sodium salt of 3-methylbenzene-1-thioglycollic-2-carboxylic acid, cooled and strongly acidified with hydrochloric acid and 4-methyl-3-hydroxythionaphthene was distilled in steam without preliminary heating. It soldifies to white needles and is quite pure for further use, m. p. 75-76°. It gradually turns on exposure to air brown-red but still it retains the property of oxythionaphthene. It remains unchanged in vacuum. It is soluble in pyridine, benzene nitrobenzene, aniline, acetic acid, amyl alcohol, acetone, chloroform, ether and is soluble in concentrated sulphuric acid with a light yellowish green colour. It crystallises from amyl alcohol in beautiful foliated needles.

The indigoid dyes described were obtained in a way similar to the isomeric 5-methyl and 6-methyl derivatives (Guha, *loc. cit.*). They were all purified by boiling with alcohol in which they are sparingly soluble and finally crystallised.

2-(4-*Methyl*)-*thionaphtheneacenaphthyleneindigo*.—It was prepared from acenaphthenequinone (0·546 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.) in glacial acetic acid (55 c.c.) and concentrated hydrochloric acid (6 c.c.). The thin needle-shaped crystalline dye (0·766 g.) was collected, purified and crystallised from acetic acid in bright silky vermillion colour, m. p. 263°. It is soluble in benzene, toluene, amyl alcohol and acetic acid. It dyes cotton in vermillion shades from an alkaline hydrosulphite vat and wool in the same colour from an acid bath. (Found : S, 9·77. $C_{21}H_{12}O_2S$ requires S, 9·75 per cent). 2-(4-*Methyl*)-*thionaphthene*-8′(3′-*chloro*)-*acenaphthyleneindigo* separated from 3-chloroacenaphthenequinone (0·6495 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.) in glacial acetic acid (65 c. c.) and concentrated hydrochloric acid (4 c. c.) as red small thread-like needles which after being washed with acetic acid and hot water were crystallised from nitrobenzene, m. p. 270-71° (with previous shrinking). It is soluble in benzene, diffiicultly soluble in acetic acid. It dyes cotton from an alkaline vat in red shades and wool in the same colour from an acid bath. (Found : Cl, 9·49. $C_{21}H_{11}O_2ClS$ requires Cl, 9·79 per cent).

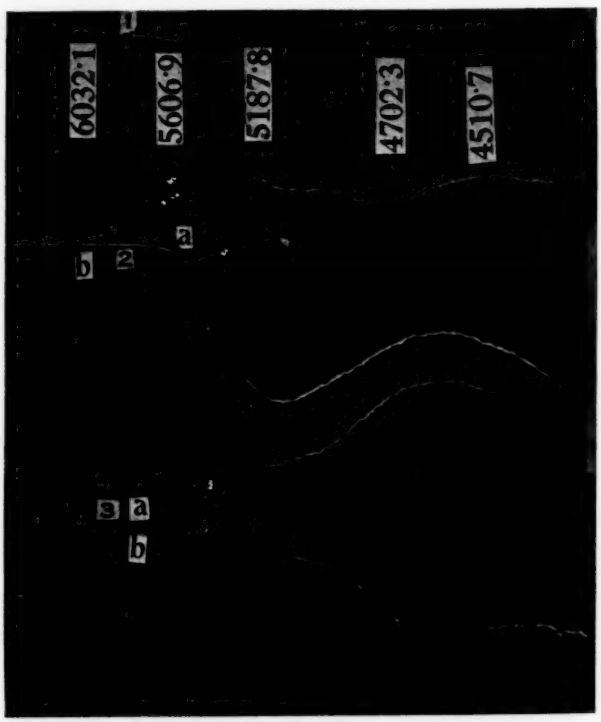
2-(4-*Methyl*)-*thionaphthene* 8′-(3′ *bromo*)-*acenaphthyleneindigo* was obtained from 3-bromoacenaphthenequinone (0·522 g.) and the methylhydroxythionaphthene (0·328 g.) in the same quantity of acetic acid and hydrochloric acid as used in the formation of the foregoing substance. The red crystalline precipitate (0·429 g.) was crystallised in the same way and it possesses properties similar to the preceding compound. It melts at 260°. The dyeing shades on cotton and on wool are similar to the last

named compound but slightly deeper. (Found : Br, 19·24. $C_{21}H_{11}O_2BrS$ requires Br, 19·65 per cent).

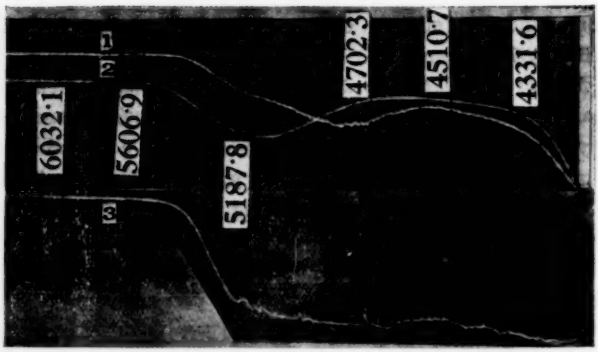
2-(4-Methyl)-thionaphthene-8′-(1′-methoxy)-acenaphthyleneindigo.—It was prepared from β-methoxyacenaphthenequinone (0·636 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.) in acetic acid (120 c.c.) and concentrated hydrochloric acid (5 c. c.). The dye (0·5265 g.) was crystallised from pyridine in fine hair like needles not melting below 305°. It is difficultly soluble in benzene and acetic acid. It dyes cotton in faint pink colour from an alkaline vat and wool in deep red shades from an acid bath. (Found : S, 9·33. $C_{22}H_{14}O_3S$ requires S, 8·93 per cent).

My thanks are due to Mr. K. Prasad and Dr. A. C. Sircar for taking interest in this work and to Messrs D. K. Bhattacharya and B. N. Ghosh for helping me readily with spectrograph and microphotometer.

FIG. 1.

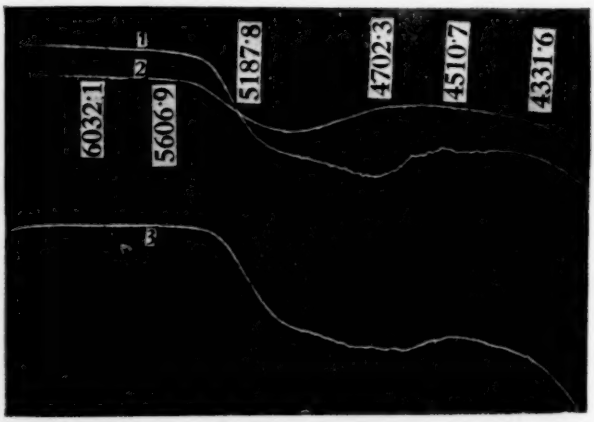


Curves 1—3 refer respectively to 2-(4-methyl)-, 2-(5-methyl) and 2-(6-methyl)-thionaphthene-acenaphthylene-indigos; corresponding exposure being (1) 17″, (2 a) 15″, (2 b) 20″ and (3 a) 10″, (3 b) 15″.



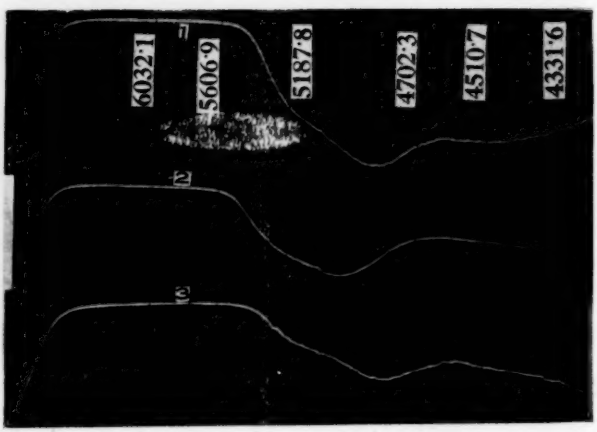
Curves 1–3 refer respectively to 2-(4-methyl)-, 2-(5methyl)- and 2-(6-methyl)-thionaphthene-8′ (3″-chloro)-acenaphthylene-indigos.

FIG. 2.



Curves 1—3 refer respectively 2-(4 methyl)-, 2-(5-methyl) and 2-(6 methyl)-thionaphthene-8′-(3-bromo)-acenaphthylene-indigos.

FIG. 3.



Curves 1—3 refer respectively to 2-(4-methyl)-, 2-(5-methyl)- and 2-(6-methyl)-thionaphthylene-8′ (1″-methoxy)-acenaphthylene-indigos.

FIG. 4.

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ON THE ANTHOCYANIN PIGMENT IN THE RIND OF SUGAR-CANE (*PURPLE MAURITIUS*).

By C. J. Dasa Rao, D. G. Walawalkar and B. S. Srikantan.

The colouring matter in the rind of *Purple mauritius* cane, which persists in the sugar, has been isolated and has been identified to be a diglucoside of the monomethyl ether of delphinidin, possibly ampeiopsidin. It has an absorption band in the region 4400-4800Å.

Considerabie doubt exists as regards the nature of the soluble colouring matter in the rind of the purple cane and which being not easily removed from the cane juice, gives rise to difficulties during the refining of the sugar prepared from the cane. Harloff (*International Sugar Journal*, 1914, **16**, 116) and Muller (*ibid.*, p. 224) identified it to be an anthocyanin from its colour reaction. Brewster (*ibid.*, 1923, **25**, 593) does not agree with this view. Sakuma and Memosa (*J. Soc. Chem. Ind.* Japan, 1935, **38**, 161, 224, 293) working with the rind of the Formosan cane, called "*Ancha*" have obtained the anthocyanin.

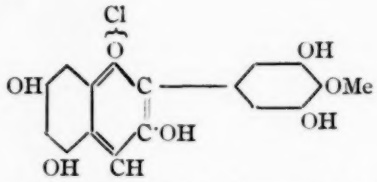
No attempt has been made so far to isolate the pigment in a pure form and identify it with one or more of the well known anthocayanins. The present investigation was undertaken with a view to isolate the colouring matter in a pure state and to elucidate its nature.

A preliminary colour reaction of the water extract of the rind by the method of Wheldale (*Biochem. J.*, 1913, **7**, 87) showed that the substance belongs to the Weinroth group (*cf.* Weigert, *Jahres Bericht*, Wien, 1895). The pigment from the rind of the cane has been extracted with glacial acetic acid, precipitated and recrystallised as red microscopic crystals from methyl alcoholic hydrogen chloride. The analytical data show the substance to be a glucoside of the formula $C_{28}H_{31}O_{17}Cl,4H_2O$. This is shown to be a diglucoside by hydrolysis with 20% hydrochloric acid. The anthocyanidin chloride then should be $C_{16}H_{12}O_7$, HCl. However, the anthocyanidin sulphate ($C_{16}H_{12}O_7$, H_2SO_4, H_2O) was prepared by the method of Willstätter and Bolton (*Annalen*, 1915, **408**, 42).

The Zeisel estimation showed the presence of only one methoxy group and the colour reactions of the demethylated product, when compared with those of the pelargonidin, cyanidin and delphinidin, clearly show that the substance is a delphinidin derivative. This evidence is fairly conclusive in the absence of alkali fusion and further study due to the insufficient quantity of the substance. Hence the anthocyanidin sulphate is a monohydrate of monomethylether of delphinidin sulphate.

There are five possible structurers for the monometeyl ethers of delphinidin. The way of distinguishing these ethers is by alkali fusion of the demethylated product. The substance was too small for such

a procedure. The colour reactions agree well with those of ampelopsidin described by Willstäter and Zollinger (*Annalen*, 1916, **412**, 164, 213, 216), Willstätter and Bendic (*Annalen*, 1916, **412**, 217). These authors ascribe the following structure to ampelopsidin purely from ferric chloride reaction. The OMe-group is attached to the middle hydroxyl of the phenol residue of the molecule.



Hence it can be said with a fair degree of certainty that the anthocyanidin in the rind of the sugar-cane *purple mauritius* is a diglucoside of monomethyl delphinidin, possibly ampelopsidin, crystallising with 4 molecules of water. Willstätter and Zollinger (*loc cit.*), however, describe a monoglucoside of ampelopsidin, while they do not deny the possibility of a diglucoside being hydrolysed to monoglucoside during the process of preparation. The authors believe, however, further work is necessary before these tentative conclusions can be affirmed. The anthocyanin from *purple mauritius* is referred to in this work as *Mauritinin*.

Experimental.

Water Extract : Colour Reactions.—Mature canes possessing a dark violet tinted rind were taken and the epidermal layer was scraped by means of a piece of glass. The aqueous extract of the scrapings were red in colour and turned deep red in hydrochloric acid, dark green with sodium hydroxide and yielded a blue-green precipitate with neutral lead acetate. 0·10 G. of the pigment was obtained as a red powder in its non-glucosidal form, starting from 25 lbs. of cane. The substance was sparingly soluble in water and in dilute acid a crimson-red colour was obtained. Caustic soda and lime water gave a green colour, but with excess of the latter the colour was destroyed. Lead acetate produced a green precipitate. Absolute alcohol dissolved it giving brown-red solution. The red colour in mineral acid was taken up by amyl alcohol layer when shaken up with amyl alcohol.

Isolation of Mauritinin Chloride.—650 lbs. of mature *purple mauritius* cane were scraped and the scrapings (about 8 lbs.) put into 5 kg. of glacial acetic acid and allowed to stand for 7 days. The extract had a deep red colour with a faint green fluorescence. To each 500 c.c. of the extract

50 c.c. of methyl alcoholic hydrochloric acid (8%) was added and the pigment was precipitated by the addition of 1 litre of ether.

The impure product was dissolved by boiling for a short time in 2% methyl alcoholic hydrochloric acid, filtered warm to remove insoluble impurities and the filtrate mixed with ¼ its volume of aqueous hydrochloric acid, when the glucoside is not hydrolysed even on long standing. On cooling the chloride separated as a violet-red precipitate which was filtered and the precipitate again dissolved in methyl alcoholic hydrochloric acid and reprecipitated by aqueous hydrochloric acid. The precipitate was filtered and washed free of chloride and dried on a porous plate.

It is brown-red in colour, soluble in alcohol giving a purple solution. On dilution it isomerises to the pseudo-base of yellow colour. An acid solution treated with sodium carbonate yields violet colouration. Ferric chloride in alcoholic solution gives a violet colour and lead acetate a light blue precipitate.

The dehydrated product has an approximate M.W. (Rast) of 702. The dehydrated anthocyanin chloride gives on complete hydrolysis 50·08% of glucose. [Found : C, 49·27 ; H, 5.76 ; Cl, 5·18. Monoglucoside of ampeolpsidin ($C_{22}H_{23}O_{12}Cl$) requires M. W., 514; glucose, 35·02% ; C, 51·36 ; H, 4·47 ; Cl, 6·18 per cent. Diglucoside of ampelopsidin ($C_{28}H_{33}O_{17}Cl$) requires M.W., 676; glucose, 53·25%; C, 49·70 ; H, 4·88, Cl, 5·18 per cent]. The percentage of water in the air-dried sample, found by vacuum drying, is found to be 9·15%. On calculation it is seen that 9·62% of water are contained in $C_{28}H_{33}O_{17}Cl$, $4H_2O$. The percentage of hydrogen is noticeably in excess perhaps due to incomplete dehydration of the substance in the last stages.

Hydrolysis of Mauritinin chloride and the Determination of Glucose.—A weighed quantity of chloride was hydrolysed by boiling with 10 c.c. of 20% hydrochloric acid (*cf.* Willstätter and Meig, *Annalen*, 1915, **408**, 61) and the mixture at once cooled. The solution was neutralised with sodium hydroxide and the glucose estimated by the method of Scales (*J. Biol. Chem.*, 1915, **23**, 61). A number of determinations were made with duration of hydrolysis varying from 3 to 7 minutes. As seen from below complete hydolysis is obtained in 5 minutes.

3 min.	hydrolysis gave	23·98%
4		42·50
5		50·00
7		50·08

Isolation of the Mauritinidin Sulphate.—10 lbs. of the scrapings from 800 lbs. of mature *purple mauritius* were collected in a large glass jar containing 1 lb. of glacial acetic acid. The method of Willstätter and

Bolton (*loc. cit.*) was followed and the lead salt was hydrolysed by 7% sulphuric acid. A brown-red powder was obtained which was difficult to crystallise, yield 0·6508 g.

It does not melt even at 300°. It forms an orange solution in alcohol giving yellow colouration with sodium carbonate and red with acids. Ferric chloride gives a faint green colour turning to violet. Caustic soda produces a permanent blue colour. An alcoholic solution containing 0·009 g. in 500 c.c. of absolute alcohol with a cell of 10 mm. thickness showed a strong band in indigo-blue region 4400-4800Å with diffuse ends. The substance when heated with nitric acid at 320° in a sealed tube and treated with $BaCl_2$ showed the presence of sulphate radicle. Loss of moisture in the air-dried sample at 100° under vacuum is 5%, giving 1 mol. of water in $C_{16}H_{12}O_7$, H_2SO_4, H_2O. The approximate M. W. of the dehydrated substance by Rast's method is 434. The substance was found to be a monomethoxy derivative, the dehydrated substance giving 7·93 and 7·79% of methoxyl groups in two Zeisel determinations. Monomethyl ether of delphinidin sulphate ($C_{16}H_{12}O_7$, H_2SO_4) requires 7·38% and the dimethyl ether of delphinidin sulphate ($C_{17}H_{14}O_7$, H_2SO_4) requires 12·15%. The slightly higher values for OMe is usual in most of monomethyl ethers of the delphinidin group, perhaps due to traces of dimethyl ether as impurity (*cf.* Perkin and Everest, " The Natural Organic Colouring matters " 1918, p. 315).

The Demethylated Mauritinidin Chloride.—The residue after estimation of the methoxyl group was warmed with a little acetic anhydride, diluted with ether and filtered. The solid was dissolved in 25% alcohol and to it was added a few drops of concentrated hydrochloric acid with a small speck of silver chloride in it. Hydrochloric acid (20%, 10 c c.) was added to it and the alcohol was slowly evaporated on a water-bath. The solution was set aside for crystallisation. Needle-shaped violet crystals with a metallic lustre were obtained. It does not melt below 350°. It is highly soluble in methyl and ethyl alcohols giving purple-red solutions. Ferric chloride with alcoholic solution of the pigment gives a stable dark blue-green colour which changes to faint violet on dilution with water. On addition of warm water to alcoholic solution, the colour fades (leuco base) but soon a pale violet precipitate settles down. From the acid solution the pigment was completely taken up by amyl alcohol. Sodium carbonate produces a violet colour which turns blue on dilution. Lead acetate gives a violet precipitate. The amount of substance was too small for any further quantitative investigation. This work was done in the Technology laboratories of the Andhra University, Waltair.

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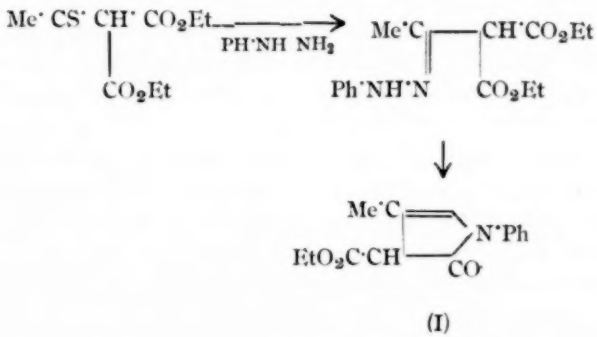
THIOKETONIC ESTERS. PART V.

By S. K. Mitra.

Synthesis of some unpolymerised β-thioketonic esters has been achieved. Some reactions characteristic to these compounds comparable with those of the corresponding oxygen analogues have been studied. The structures of sodiothioketonic esters has also been confirmed conclusively.

The formation of unpolymerised sulphur analogues from the reaction of hydrogen sulphide with ketonic compounds *e.g.* β-ketonic esters, possessing marked tendency of enolisation has been suggested in a previous communication (Mitra, *J. Indian Chem. Soc.*, 1933, **10**, 71). The present investigation deals mainly with the general applicability of the reaction and a study of the methylene group activated by the thiocarbonyl group thus introduced.

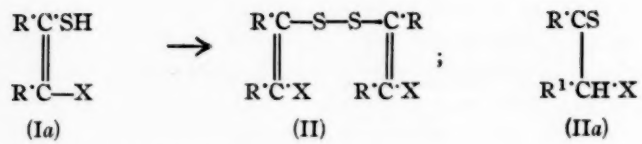
Diethyl thioacetylmalonate has been synthesised from the interaction of hydrogen sulphide and the corresponding oxygen analogue. Its constitution has been established by the synthesis of 1-phenyl-3-methyl-4-carbethoxy-5-ketopyrazolone (I) from the reaction of the thioketo-ester with phenylhydrazine.



(I)

Ethyl methylthioacetoacetate (Ia) in unpolymerised state, together with another material, has been isolated from the reaction of hydrogen suiphide with ethyl methylacetoacetate. The latter compound does not possess any thiol or thioketonic group. Structure (II) has been suggested for it.

Evidently the compound was formed through oxidation of two molecules of the thioketo-ester in thiol phase.

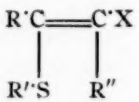


(Ia) (II) (IIa)

(R = Me ; X = CO_2Et); R^1 = Me, Me>CH·CH_2

Ethyl *iso*butylthioacetoacetate (II*a*) has also been synthesised in a similar manner.

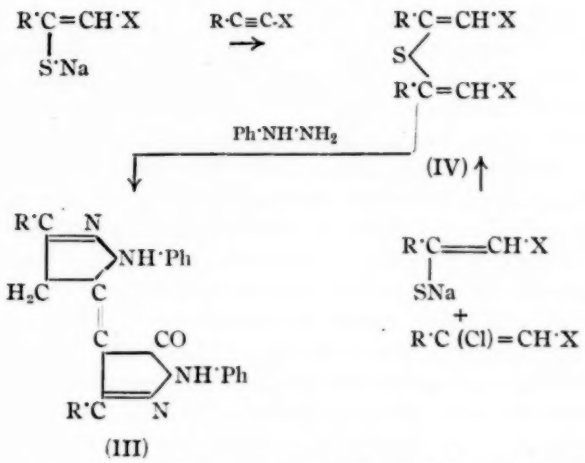
All these β-thioketonic esters react with metallic sodium like the corresponding oxygen analogues and can be alkylated by the action of alkyl halides on the corresponding sodio derivatives. These alkylated products are *S*-ethers (*vide* Mitra, *loc. cit.*) and the structure of these compounds must be regarded as



(R′ = alkyl or aryl ; R″ = H or any group).

An attempt to synthesise secondary thiols from the interaction of β-thioketonic esters and alkylmagnesium halides failed. The reaction gave rise to a hydrocarbon and the magnesium halide compound of the thioketonic ester. The latter compound on treatment with mineral acids liberated the thioketonic ester unchanged.

It was next thought desirable to study the structure of the sodio derivative in order to elucidate the mechanism of alkylation. Any information of this kind obviously necessitates a study of the addition of the sodio derivative on an unsaturated system in Michael's way. An attempt to condense sodio ethyl thioacetoacetate with ethyl cinnamate failed. A similar reaction with ethyl tetrolate furnished a compound which does neither possess a thiol nor a thioketonic group. Its reaction with phenylhydrazine furnished 1-phenyl-3-methyl-5-ketodehydropyrazolone (III, Mohr, *Ber.*, 1905, **38**, 2578) thus giving a proof of the presence of acetoacetic residue in the molecule. The structure (IV) which has been suggested for the compound has been confirmed by its synthesis from the interaction of sodio ethyl thioacetoacetate and ethyl β-chlorocrotonate.



(III)

Thus it appears conclusive that the sodio derivative of β-thioketonic esters mainly exists in $\rangle C-S\cdot Na$ phase unlike β-ketonic esters and a simple replacement of sodium with alkyl group explains the formation of *S*-ethers. If the addition of sodio derivative depend upon the tendency of saturation of the unsaturated system, then the failure of ethylene derivatives to form any additive products is obvious. The influence of the unsaturated system in ethyl cinnamate on the sodium atom in the mercaptide phase of the thio-ester is of the order of what it (sodium) is already under, due to the presence of a double bond in the sodio derivative itself and hence is not sufficient to effect any addition. Thus the capability of β-ketonic esters to react with the unsaturated compounds of the ethylene series and the formation of *C*-ethers on alkylation clearly prove the structure of their sodio-derivative as $>CH\cdot Na$.

EXPERIMENTAL.

Ethyl Thioacetylmalonate.—Diethyl acetylmalonate (15 g.) was dissolved in absolute alcohol (50 c.c.) saturated with dry hydrogen chloride. Hydrogen sulphide was passed through the solution (24 hours at 15°). The mixture was poured over ice and extacrted with benzene (200 c.c.). The benzene solution was washed with sodium carbonate, dried and distilled at 120° / 4 mm. (Found : C, 49·6 ; H, 6·6 ; S, 14·3. $C_9H_{14}O_4S$ requires C, 49·5 ; H, 6·4 ; S, 14·6 per cent).

1-Phenyl-3-methyl-4-carbethoxy-5-ketopyrazolone.—Diethyl thioacetyl-

malonate (4 g.) was mixed with phenylhydrazine (2 mol. per 1 mol. of ester). When the evolution of hydrogen sulphide moderated, the mixture was heated at 100° for 8 hours. The product after washing with ether furnished colourless needles from acetic acid, m.p. 121-22°. It was identified in the usual manner. It gave a violet colouration with ferric chloride.

Ethyl Methylthioacetoacetate.—Hydrogen sulphide was passed (18 hours) through a solution of ethyl methylacetoacetate (100 g.) dissolved in alcohol (200 c.c.) saturated with dry hydrogen chloride. The oil which separated on pouring the mixture over ice was extracted with benzene. The fraction, b.p. 78°-104°/10 mm. was collected from the benzene solution. This was dissolved in ethyl alcohol (100 c.c.) and while the solution was stirred, freshly precipitated lead oxide was slowly added. The yellow lead salt was collected and washed with alcohol. The mercaptide was decomposed with dilute hydrochloric acid and the liberated ester was extracted with benzene (500 c.c.). The fraction, b.p. 95°/10 mm. was isolated from the benzene solution. (Found : C, 52·2 ; H, 7·6 ; S, 19·5. $C_7H_{12}O_2S$ requires C, 52·5 ; H, 7·5; S, 20·0 per cent).

Ethyl iso*Butylthioacetoacetate.*—Hydrogen sulphide was passed (24 hours) through a solution of ethyl *iso*butylacetoacetate (25 g.) in ethyl alcohol (100 c.c.) saturated with dry hydrogen chloride. The mixture was extracted, purified in the usual manner as stated above and the ester was isolated as an oil, b.p. 122°/5 mm. (Found : C, 59·2 ; H, 9·1 ; S, 15·3. $C_{10}H_{18}O_2S$ requires C, 59·4 ; H, 8·9 ; S, 15·8 per cent). Freshly distilled thioketonic esters are rose-red liquids possessing sweetish smell. The colour deepens with rise of temperature. Phenylhydrazine reacts with them at the room temperature (15°) with liberation of hydrogen sulphide. Alcoholic ferric chloride gives a violet colouration and alcoholic iodine is rapidly decolourised. They form coloured lead salt and react with metallic sodium vigorously with evolution of hydrogen.

Ethyl β-Methylmercapto-α-methylcrotonate.—Ethyl methylthioacetoacetate (8 g.) was added to ethyl alcohol (50 c.c.) containing sodium (1 g.). Methyl sulphate (6 g.) was next added to the mixture which was boiled under reflux (4 hours). The product on treatment with excess of water was extracted with benzene. The fraction b.p. 225°/750 mm. was collected from the benzene solution. (Found : C, 54·9 ; H, 8·2 ; S, 18·2. $C_8H_{14}O_2S$ requires C, 55·1 ; H, 8·0 ; S, 18·3 per cent).

Ethyl β-Methylmercapto-α-methylcrotonate (5 g.) was boiled with aqueous hydrochloric acid (*d* 1·19, 25 c.c. diluted with 25 c.c. of water). The liberated gases were passed through washers containing alcoholic iodine and baryta. Methyl disulphide was identified in the iodine solution.

The liberation of methylmercaptan confirms the *S*-ether structure of the alkylated products.

Ethyl β-Ethylmercapto-α-methylcrotonate.—Ethyl methylthioacetoacetate (13 g.) was added to emulsified sodium (2 g.), suspended in dry benzene (50 c.c.). After keeping the mixture at the room temperature (15°) for 2 hours, ethyl iodide (16 g.) was added and the mixture boiled under reflux (6 hours). The thio-ether was isolated as an oil, b.p. 220°/7 mm. (Found: C, 57·0 ; H, 8·6. $C_9H_{16}O_2S$ requires C, 57·4 ; H, 8·5 per cent).

Ethyl β-Methylcarbethoxymercapto-α-methylcrotonate.—Ethyl monochloroacetate (13 g.) was added to sodio derivative of ethyl methythioacetoacetate (ester, 18 g ; sodium, 3 g.) in benzene suspension and the mixture was boiled under reflux (5 hours). The thio-ether was isolated as an oil, b.p. 160°/6 mm. (Found : C, 53·3; H, 7·5. $C_{11}H_{18}O_4S$ requires C, 53·6; H, 7·3 per cent).

Diethyl Methyldithioacetoacetate (II).—Hydrogen sulphide was passed (12 hours) through a solution of ethyl methylacetoacetate (20 g.), dissolved in alcohol (100 c.c.) saturated with hydrogen chloride. The mixture was left at the room temperature (15°) for 3 days, poured over ice and extracted with benzene. After the removal of the low boiling fraction, mainly consisting of unpolymerised ethyl methylthioacetoacetate and its unreacted oxygen analogue, a fraction, b.p. 180°/6 mm., was collected. The same product was isolated by oxidation of unpolymerised ethyl methylthioacetoacetate with alcoholic iodine. (Found : C, 52·6 ; H, 7·1; S, 20·0. $C_{14}H_{22}O_4S_2$ requires C, 52·8 ; H, 6·9 ; S, 20·1 per cent).

Ethyl β-Methylmercaptoglutaconate.—Dimethyl sulphate (6 g.) was added to sodio derivative of ethyl thioacetonedicarboxylate (ester, 20 g.; sodium, 2·1 g.) and the mixture was boiled (4 hours). The thio-ether was isolated as an oil, b.p. 135°/6 mm. (Found : C, 51·6 ; H, 6·8 ; S, 14·0. $C_{10}H_{16}O_4S$ requires C, 51·7 ; H, 6·8 ; S, 13·7 per cent).

Ethyl β-Acetylmercaptoglutaconate.—A mixture of ethyl thioacetonedicarboxylate (20 g.) and acetic anhydride (50 c.c.) containing pyridine (1 c.c.) was boiled under reflux (4 hours). The mixture was poured into ice and extracted with ether. The fraction, b.p. 150°/9 mm., was isolated from the ethereal extract. (Found : C, 50·4 ; H, 6·1. $C_{11}H_{16}O_5S$ requires C, 50·7 ; H, 6·1 per cent).

Ethyl β-Methylcarbethoxymercaptoglutaconate.—Ethyl monochloroacetate (12 g.) was added to sodio ethyl thioacetonedicarboxylate (ester, 19 g., sodium, 2 g.) in ethyl alcohol (40 c.c.) and the mixture was boiled. The compound was obtained as an oil, b.p. 170°/6 mm. (Found : C, 51·2 ; H, 6·8 ; S, 10·2. $C_{13}H_{20}O_6S$ requires C, 51·3 ; H, 6·6 ; S, 10·5 per cent).

Action of n*-Butylmagnesium Iodide on Ethyl Thioacetondicarboxylate.*—Ethyl thioacetonedicarboxylate (5 g.) was added to *n*-butyl magnesium iodide (Mg, 1 g.; butyl iodide, 6 g.) in ether solution. The pasty yellow mass, which separated, was washed several times with ether. The material on treatment with aqueous hydrochloric acid liberated ethyl thioacetonedicarboxylate unchanged. During the addition of ethyl thioacetonedicarboxylate to butylmagnesium halide, a copious evolution of a gas took place. This was probably butane.

The Formation of (IV). *(i) By Addition of Sodio ethyl thioacetoacetate to Ethyl Tetrolate.*—Ethyl thioacetoacetate (23 g.) in dry benzene (50 c.c.) was added to emulsified sodium (4·1 g.), suspended in the same solvent (200 c.c.). After allowing the mixture to stand (6 hours) at the room temperature (15°), ethyl tetrolate (20 g.) was added and the whole mass was boiled under reflux (5 hours). It was then treated with ethyl alcohol (40 c c.) and diluted with excess of hydrochloric acid. From the benzene solution the compound was isolated as an oil, b.p. 155°/12 mm. (Found : C, 55·8 ; H, 7·1; S, 12·2. $C_{12}H_{18}O_4S$ requires C, 55·8; H, 7·0 ; S, 12·4 per cent).

(ii) By the Action of Ethyl β-chlorocrotonate on Sodio ethyl thioacetoacetate.—Ethyl thioacetoacetate (10 g.) was added to a solution of sodium (2 g.) in alcohol (50 c.c.). The mixture was then treated with ethyl β-chlorocrotonate (10 g.) and boiled under reflux (5 hours). The product was diluted with excess of water and extracted with benzene. The fraction, b.p. 158°/15mm., from the benzene solution was collected. (Found : S, 12·3. $C_{12}H_{18}O_4S$ requires S, 12·4 per cent).

Action of Phenylhydrazine on Compound (IV, R=Me) *and the Formation of 1-Phenyl-3-methyl-5-ketodehydropyrazolone* (III, R = Me). —Ethyl (ethyl β-crotonate)-mercaptocrotonate (IV, 5 g.) was treated with phenyhydrazine (3 g.) and boiled under reflux (1 hour). The product was digested with alcohol and the residue furnished colourless needles of pyrazolone from acetic acid, m.p. 320° (decomp.).

My thanks are due to Prof. Samuel Smiles, F.R.S. for his kind interest in this investigation and for placing the resources of his reasearch laboratory at my disposal.

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THE DETERMINATION OF THE PARACHORS OF INORGANIC SALTS IN SOLUTIONS AND THEIR STRUCTURE. PART I. POTASSIUM SALTS.

JAMIAT V. LAKHANI AND RUSTOM P. DAROGA.

Hammick and Andrew's equation for calculating the parachor of mixtures of two liquids has been modified to apply to the calculation of parachor of solid inorganic salts in solution.

The parachor values of 15 salts found in many cases are in good agreement with the calculated $\Sigma[P]$ of the salts concerned.

The atomic parachor of potassium is found to be (110) in the case of monovalent salts and (106) when the salt is bivalent containing two atoms of potassium.

The parachor values of the 9 potassium salts : carbonate, bicarbonate, pyrosulphate, chromate, chlorate, bromate, perchlorate, cyanide and sulphocyanide which have not been previously determined are now found by this method.

Hammick and Andrew (*J. Chem. Soc.*, 1929, 754) have shown that the parachors of substances unstable in the pure liquid state can be calculated from the surface tensions and densities of their solutions in solvents of known parachor on the assumption that the parachors of solvent and solutes are additive. Thus parachors of the solutes have been calculated by finding the parachors P_m of a solution containing a molecular fraction x of the solute, in a solvent of known parachor, by means of the equation.

$$P_m = (1-x)\,P_p + x\,P_x$$

where P is the unknown parachor of the solute to be determined, P_p is the parachor of the solvent (already known), and x is the molecular fraction of the solute.

The " straight line mixture law " of Hammick and Andrew (*loc. cit.*) has been found in several cases to be independent of dilution. In other cases, where the value of the solute parachor P_x changes with the dilution, the parachor of the pure solute has been deduced from a series of values of P_x by straight line extrapolation.

Ray (*J. Indian Chem. Soc.*, 1934, **11**, 671) pointed out the applicability of the 'straight line mixture law' in the determination of parachor of organic solids dissolved in liquids. But in all this and subsequent work no attempt has been made to determine the parachor of solid inorganic substances in solution and of such other salts which are difficult to fuse or which decompose before fusion.

The authors have kept the above object in view in their investigations and have shown from the experimental work that by modification of the Hammick and Andrew's equation, the parachor of very many inorganic salts and elements, which have not been previously determined, could be accurately calculated from the measurement of density and surface tension of their solutions.

In the true solutions or completely miscible mixtures, the solute (liquid or solid) is believed to be dispersed in the solvent in the molecular or ionic condition, and therefore in solution the usual distinction between the liquid state and the solid state entirely disappears. There is one difference, however, that when two liquids are mixed (or one dissolved into the other) the total volume of the mixture is generally equal to the sum of the individual volumes of the two components. But when a solid is dissolved in a solvent the volume of the solution in many cases (neglecting a small change in volume) is practically identical with the volume originally taken. Therefore, since according to Sugden's equation,

$$P = V.\gamma^{\frac{1}{4}}$$

where V is the molecular volume, or as in Hammick and Andrew's equation (*loc. cit.*)

$$P_m = V_m.\gamma^{\frac{1}{4}}$$

where V_m is the mean molecular volume, the parachor values calculated by the above equation, when inorganic salts are dissolved in the solvent (water), will be half as when liquids are dissolved. Consequently Hammick and Andrew's equation, when applied to the inorganic salt solutes in solution and when volume changes are negligible, should be written as

$$P_m = (1-x)\,P_p + x\frac{P_x}{2}.$$

The measured values of the parachor, obtained with a variety of salts dissolved in water, indicate that the mixture law, as modified in accordance with the above view, correctly holds and the values obtained are also identical with those calculated from the atomic and structural parachors of the compounds concerned.

The parachor is found to be independent of the concentration up to the lower limit of 0·009 g. mol. of the solute per 100 g. of the solvent. The more dilute solutions containing less than 0·009 g. per 100 g. of the solvent were not found to yield the correct parachor values.

With such solutions of solids, where the volume changes take place to a considerable extent, the value of P_x changes with the concentration and the parachor of the solute is then deduced from a series of the values

of P_x by straight line extrapolation as pointed out by Hammick and Andrew (*loc. cit.*).

EXPERIMENTAL.

In this investgation the surface tension has been measured by the double capillary rise method in the manner described by Sugden (*J. Chem. Soc.*, 1921, **119**, 1483).

All determinations were made at a constant temperature of $30° \pm ·2$ maintained in a self regulated thermostat. Extra pure salts of Merck or Kahlbaum have been used throughout.

The solutions were made by dissolving a weighed quantity of an anhydrous salt in 100 g. of distilled water thus ensuring the same content of water in all solutions. If the solution is made up to a definite volume, the water content of the solution will vary according as the solution contracts or expands (Mogan and McKirahan, *J. Amer. Chem. Soc.*, 1913, **35**, 1760). Great care was taken to expose the solution as little as possible to the atmosphere to avoid any fine stray dust particles from getting into the solution.

In those cases, where the anhydrous salt was not available or the water of crystallisation could not be completely removed or the solubility of the salt was considerably altered on becoming anhydrous, the hydrated salt was used for making the solution, but the weight of the water of crystallisation was subtracted from the total weight of the salt, and the same added to the quantity of water used as solvent.

The densities of solutions were measured at $30° \pm ·2$ by using a specific gravity bottle of 10 c.c. capacity.

For calculating the sum of the atomic parachors, use has been made of the atomic and structural parachors, given in Sugden's book ("The Parachor and Valency," pp., 38, 131, 181).

The results of 15 potassium salts are recorded in columns 1 to 10 of the following table.

A reference to columns 7 and 8 indicates that the figures of the measured parachor agree very closely with those of the calculated parachor, except in the case of potassium carbonate. The deficit in this case is probably due to the formation of a stable hydrate of carbonate in contact with water. (*cf.* Mellor, "Modern Inorganic Chemistry," p. 810).

In column 9 are given for comparision the values of parachor obtained by the fusion method by Sugden and Wilkins. Only the parachors of KBr and $K_2Cr_2O_7$ agree with the calculated values. In other cases the experi-

TABLE I.

Results obtained with solutions of different potassium salts.

Substance.	X (Molar fraction of solute)	D (Density of solution).	γ (Surface tension of the soln.).	M_m (Mean mol. wt.)	P_m (Measured parachor of the soln.).	P_x (observed parachor of the solute compound).	$\Sigma[P]$ (Calc. parachor of the solute compound).	Parachor of the compound observed by Sugden and Wilkins by fusion method.†	Parachor of K used in calculating $\Sigma[P]$.
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
KCl	0·05882	1·135	76·75	21·312	55·56	161·2			
	0·06301	1·150	77·91	21·565	55·69	161·9 (mean 161·8)	162·7	156·6	110
KBr	0·04342	1·188	75·39	22·386	55·49	174·48	176·4	174·3	110
KI	0·03682	1·225	71·61	23·451	55·67	200·0	199·4	205·2	110
KNO_3	0·04266	1·130	74·47	21·538	55·98	201·3	200·9	189	110
K_2CO_3 (anhydrous)	0·03765	1·201	87·67	21·516	57·35	286·4	293·6	Not done	106
$KHCO_3$	0·03474	1·106	76·86	20·854	55·84	214·2	208·7	Not done	110
K_2SO_4	0·01227	1·074	79·29	19·914	55·35	329·2	330·6	328·0	106
K_2CrO_4 (at 35°)	0·02702	1·208	84·03	22·761	57·07	335·4	336·8	Not done	106

TABLE I (contd.).

Substance.	X (molar fraction of solute).	D (Density of solution).	γ (Surface tension of the soln.)	M_m (Mean mol. wt.).	P_m (Measured parachor of the solution).	P_x (observed parachor of the solute comp.).	$\Sigma[P]$ (Calculated parachor of the solute comp.).	Parachor of the compound observed by Sugden and Wilkins by fusion method.†	Parachor of K used in calculating $\Sigma[P]$.
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
$KClO_3$*	0·02157	1·089	68·05	20·252	53·4	218·0	217·9	Not done	110
$KBrO_3$*	0·01592	1·104	68·83	20·368	53·19	231·2			
	0·02111	1·138	68·93	21·145	53·53	233·0	231·6	Not done	110
						(mean 232·1)			
$KClO_4$*	0·01029	1·043	67·75	19·235	52·86	237·2	236·3	Not done	110
KCN	0·06465	1·097	73·14	21·048	56·08	172·36	172·3	Not done	110
KCNS	0·03574	1·085	72·36	20·832	55·98	218·8	218·9	Not done	110
$K_2Cr_2O_7$	0·009101	1·096	77·76	20·505	55·55	450·6	451·2	450·8**	106
$K_2S_2O_7$	0·02080	1·201	82·56	22·918	57·45	439·4	438·8	Not done	106

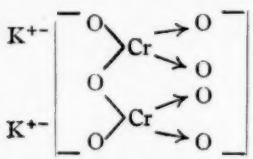
* As these salts are not sufficiently soluble at the ordinary temperature to contain the required molar fraction of the solute, they were determined at the temperature of 60°.

** (*J. Chem. Soc.*, 1928, 263).

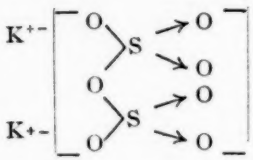
† (*J. Chem. Soc.*, 1929, 1291).

mentally determined values of the parachor by fusion method are either too high or too low. It is also seen that the solution method measures the parachor very accurately of the salts like $KClO_3$, $KBrO_3$, $KClO_4$, KCN, KCNS, K_2CrO_4, and $K_2S_2O_7$, which decompose before fusion.

In calculating $\Sigma[P]$ of $K_2Cr_2O_7$ and $K_2S_2O_7$, the structures given below have been found to fit in with the measured parachor values.



Potassium dichromate.



Potassium pyrosulphate.

In column 10, it will be observed that in calculating the $\Sigma[P]$ of monovalent salts, the figure (110) as the atomic parachor of potassium has been used. This value has been deduced by Sugden from the study of several salts in their fused state (*J. Chem. Soc.*, 1929, 1291). On the other hand in calculating $\Sigma[P]$ of the bivalent salts, the value of (106) for the atomic parachor has been employed. This value again is that deduced from Jaeger's data of surface tensions and densities of halides and sulphates of potassium (*Z. anorg. Chem.*, 1917, **101**, 1). In computing the $\Sigma[P]$ of potassium dichromate, Friemann and Sugden (*J. Chem. Soc.*, 1928, 263) have made use of the constant (106) for potassium. In this connection it will be of interest to mention that the observed parachor of $K_3Fe(CN)_6$, leads to the parachor value of (110) for potassium and that of $K_4Fe(CN)_6$ gives the value of (106) as the atomic parachor for the metal. It thus appears that when odd number of potassium atoms are present in a salt the parachor constant equals to (110), whilst when the salt contains an even number of potassium atoms, the parachor corresponds to (106). It is difficult to assign any explanation for this pecularity at present, nor is there any conclusive evidence in the case of other monovalent atoms showing similiar behaviour.

The "solution method" is being extended to the determination of parachor of several soluble inorganic salts of sodium, lithium, rubidium, magnesium, strontium, barium, manganese, iron, cobalt, nickel, etc., the results of which will be published in subsequent papers. The atomic parachor of the metal is also deduced therefrom.

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DETERMINATION OF PARACHOR IN SOLUTION. PART II. PARACHOR OF INORGANIC SALTS IN AQUEOUS SOLUTION.

By Susil Kumar Ray.

Parachors of some simple inorganic salts were determined in solution in order to find out the effect of ionisation, if any, on the parachor values and to establish, if possible, a method for the determination of parachor of compounds which are ionised in solution.

It was found that the parachors gradually increase with the increase of concentration and ultimately become nearly constant at a high concentration. From the results obtained it appears that in the study of inorganic salts in aqueous solution the atomic parachor constants can not be employed but a different set of values for the ions are required.

Parachors of some simple inorganic salts were determined in aqueous solution, in order to find out the effect of ionisation, if any, on the parachor value and to establish, if possible, a method for the determination of parachor of compounds which are ionised in solution.

In the present paper an attempt has been made to determine the parachors of ammonium chloride, ammonium bromide, ammonium nitrate, potassium chloride and potassium iodide in aqueous solution. The surface tension was determined by the method of maximum bubble pressure as in the previous work. The results are given in Tables I—V. The theoretical parachors have been calculated from the atomic constant.

In this connection it may be mentioned that Sugden and Wilkins (*J. Chem. Soc.*, 1929, 1291), published a paper dealing with the parachor of fused salts. They have employed one and the same atomic constant for the combined atom and for the ion of an element, which appears to be somewhat difficult to reconcile with modern atomic theories which imply that positive ions occupy a smaller and negative ions a larger "volume" than the corresponding atoms in a co-valent combination (*cf.* Born, *Z. Physik*, 1920, **1**, 45). Mumford and Phillips (*J. Chem. Soc.*, 1929, 2112), are of opinion that, "although the parachors so calculated are in agreement with the observed values in the case of certain salts of organic bases, the fact that large positive or negative anomalies are found with other similarly constituted salts strongly suggests that the agreement is fortuitous, and due to counterbalancing variations in the volumes of the two ions." Mumford and Phillips calculated the parachor values of the ions of the halogen from the atomic parachors of the rare gases, on the assumption that the radius of similarly constituted atoms and ions is determined by the distribution of the outermost electrons, and is inversely proportional to the effective nuclear charge. Cal-

culation on this basis, either according to the older Bohr theory or according to the newer wave mechanics (*vide* Pauling, *J. Amer. Chem. Soc.*, 1927, **49**, 765) indicates, according to these authors, that the parachor values of the halide ions should, on an average, be 1·46 times those of the atoms of the corresponding rare gases and thus about 1·44 times those of the halogen atoms in non-polar combination. The approximate ionic parachor obtained by the use of this factor, when subtracted from the observed parachor of the fused alkali metal salts, give sensively constant values for the parachors of the different alkali metal ions and from these in turn, the constants for various acidic radicles may be obtained.

It will be observed from the results given in the following tables, that the parachor gradually increases with increase of concentration and ultimately becomes nearly constant. In the case of ammonium salts, the theoretical value of the parachor is reached only with the bromide, while with the chloride and the nitrate the values are about 10 units lower. In the case of potassium chloride and iodide the observed values are found to be about 50 units lower than the calculated values.

The present work is not yet complete but the study of various other salts are under investigation. From the results already obtained and given in this paper, it appears, that in the study of parachor of inorganic salts in aqueous solutions, the atomic parachor constants cannot be employed but a different set of values for the ions are required. In this connection it may be noted that the values for the ions as deduced by Mumford and Phillips (*loc. cit.*) do not agree with the results obtained in the present investigation.

In the following tables, x denotes the mol. fraction of the solute, M_m, the mean molecular weight of the solution, P_m, the mean parachor and P_x, the parachor of the solute. The observations were carried out at the average temperature of about 30°.

TABLE I.

Ammonium chloride in water.

x.	Density.	Surface tension.	M_m.	P_m.	P_x.
0·0	0·9971	72·16	18·00	52·62	—
0·006625	1·001	72·11	18·22	52·70	107·3
0.009775	1·002	72·11	18·30	52·97	107·3
0·01113	1·007	72·45	18·39	53·28	110·5
0·01749	1·013	73·91	18·61	53·65	111·6
0·02198	1·017	73·16	18·78	53·99	115·1
0·04871	1·037	73·60	19·73	55·69	115·6
0·05977	1·044	74·30	20·12	56·57	118·6
0·07614	1·054	75·04	20·70	57·79	120·5
0·09915	1·069	75·88	21·52	59·40	121·0

The calculated parachor is 133·6.

TABLE II.

Ammonium bromide in water.

x.	Density.	Surface tension.	M_m.	P_m.	P_x.
0·0	0·9971	72·16	18·00	52·62	—
0·009935	1·027	72·06	18·79	53·33	123·8
0·01248	1·035	71·73	19·00	53·46	120·2
0·01565	1·043	73·14	19·25	53·93	136·1
0·02647	1·072	73·76	20·11	54·98	141·7
0·03350	1·093	75·63	20·70	55·86	148·6
0·03923	1·105	75·73	21·15	56.44	149·4
0·04979	1·134	76·23	21·98	57·24	145·4
0·06349	1·167	77·21	22.98	58·39	145·5
0·08132	1·210	78·43	24·51	60·32	147·3
0·1048	1·261	77·50	26·39	62·10	143·1

The calculated parachor is 147·3.

TABLE III.

Ammonium nitrate in water.

x.	Density.	Surface tension.	M_m.	P	P_x.
0·0	0·9971	72·16	18·00	52·62	—
0·02997	1·043	70·24	19·86	55·12	135·8
0·03804	1·054	70·34	20·36	55·91	138·8
0·04837	1·069	72·21	21·00	57.28	148·9
0·06187	1·085	73·77	21·84	58·99	155·5
0·07971	1·107	75·43	22·95	61·15	159·6
0·1036	1·133	77·21	24·43	63·87	161·4
0·1363	1·165	79·16	26·45	67·69	163·5
0·1826	1·206	80·63	29·32	72·88	168·6

The calculated parachor is 173·4.

TABLE IV.

Potassium chloride in water.

x.	Density.	Surface tension.	M_m.	P_m.	P_x.
0·0	0·9974	72·31	18·00	52·62	—
0·01189	1·028	73·90	18·67	53·23	103·5
0·01489	1·036	74·16	18·84	53·35	102·1
0·01866	1·044	74·22	19·05	53·56	105·5
0·02502	1·056	74·50	19·41	53·99	106·7
0·02936	1·070	75·56	19·67	54·20	105·9
0·03134	1·074	75·73	19·78	54·33	106·9
0·03941	1·091	76·47	20·24	54·81	107·8
0·04969	1·114	77·47	20·80	55·38	107·7

The calculated parachor is 164 (10).

TABLE V.

Potassium iodide in water.

x.	Density.	Surface tension.	M_m.	P_m.	P_x.
0·0	0·9974	72·31	18·00	52·62	—
0·01105	1·076	71·83	19·64	53·19	103·1
0·01386	1·094	72·93	19·98	53·35	105·3
0·02328	1·162	73·05	21·45	53·95	109·4
0·03245	1·204	71·24	22·81	55·03	127·0
0·04114	1·254	72·56	24·09	56·07	136·1
0·05248	1·314	74·27	25·76	57·51	146·0
0·06719	1·393	75·01	27·94	59·05	148·3
0·08642	1·490	74·26	30·78	60·69	145·2

The calculated parachor is 200 (10)

My grateful thanks are due to Prof. A. Maitra of the Presidency College for his kind interest in the work and to Mr. S. N. Ray for his help in the calculation of the results.

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STUDY OF THE CRYSTALS OF *P*-NITROANILINE AND *P*-NITROTOLUENE BY THE *X*-RAY SINGLE CRYSTAL ROTATION METHOD.

By Mata Prasad and R. N. Merchant.

From the rotation photographs taken about the three crystallographic axes ot single crystals of the two substances, the following dimensions of the unit cell were determined :*p*-Nitroaniline (15·31Å, 6·085Å, 8·36Å ; $\beta = 126°11'$), *p*-nitrotoluene (6·41Å, 14·10Å, 15·39Å). A large number of planes were identified on oscillation photographs and the halvings showed that the first crystal belongs to the space group C_{2h}^5 and the second to Q_h^2.

Complete structure analyses of various aromatic compounds, *e.g.*, naphthalene and anthracene (*Indian J. Phys.*, 1930, **4**, 557; *Proc. Roy. Soc.*, 1933, **A140**, 79), diphenyl (*Indian J. Phys.*, 1932, **7**, 43 ; *J. Amer. Chem. Soc.*, 1931, **53**, 167 ; *Proc. Roy. Soc.*, 1933, **A142**, 333; *Nature*, 1933, **181**, 153), durene (*Proc. Roy. Soc.*, 1933, **A141**, 594 ; 1933, **142**, 659) etc. show that the size and shape of the constituent benzene rings remain unaltered in all these compounds. This rigidity of the benzene ring is a very useful guide in the determination of the structure of any new substance. James, King and Horrocks (*Proc. Roy. Soc.*, 1936, **A153**, 225), have found, however, that the benzene ring in *p*-dinitrobenzene is slightly puckered, that carbon atoms to which the nitro groups are attached being situated about 0·1Å above or below the plane containing the other four nuclear carbon atoms. It is with an idea to find out whether the ring in similar simple benzene derivatives is plane or puckered that a study of these compounds has been started in this laboratory. The present paper gives the results of a preliminary investigation to determine the space group to which the crystals of *p*-nitroaniline and *p*-nitrotoluene belong.

p-*Nitroaniline.*

The crystals of *p*-nitroaniline belong to the monoclinic prismatic class and develop the following faces :

$r\{20\bar{1}\}$, $p\{110\}$, $q\{011\}$, $c\{001\}$.

The axial ratio is $a:b:c = 2{\cdot}5198 : 1 : 1{\cdot}4215$, $\beta = 126°11'$. (Groth, "Chemische Krystallgraphie," **V**, p. 181).

p-Nitroaniline was purified by repeated crystallisations from chloroform. Thin plates, bright yellow in colour, were obtained by a slow evaporation of the solution of the substance in chloroform. The melting point was found to be 147°.

Rotation photographs taken about the three crystallographic axes, using *X*-rays from a Shearer gas-tube fitted with copper target ($\lambda = 1{\cdot}54$ Å), give the following dimensions of the unit cell.

$$a = 15{\cdot}31\text{Å}, \quad b = 6{\cdot}085\text{Å}, \quad c = 8{\cdot}63\text{Å}, \quad \beta = 126°11'.$$

The axial ratio is $a : b : c = 2{\cdot}516 : 1 : 1{\cdot}419$ which agrees well with that given in Groth (*loc. cit.*).

Oscillation photographs were taken at suitable small angles about the *a*, *b* and *c*-axis and the planes corresponding to the spots identified with the help of Bernal's chart. The following tables (I and II) give the list of planes together with an approximate idea of their intensities for which the usual symbols have been used.

TABLE I.

Axial planes.		Prism planes (okl).		Prism planes (hol).			Prism planes (hko).	
001	w. m.	011	w. m.	201	v. w.	$\bar{2}01$	m. s.	110 m. s.
002	m. s.	012	m.	202	v. w.	$\bar{2}02$	w.	120 w. m.
020	w.	022	w. m.	203	v. w.	—		130 w.
200	m.	031	v. w.	401	w.	$\bar{4}01$	w. m.	230 v. w.
600	w.					$\bar{4}02$	m.	310 w.
						$\bar{4}03$	w.	410 m.
						404	w. m.	610 v. w.
						601	w. m.	620 v. w
						603	w.	
						802	w.	

TABLE II.

General planes.

111 m.s.	$\bar{1}11$ m.	411 w.	$4\bar{1}1$ v.w.
112 w.m.	$\bar{1}12$ w.	412 m.	$4\bar{1}2$ m.
113 w.m.	...	413 w.m.	$4\bar{1}3$ w.
121 w.m.	$\bar{1}21$ w.	...	$4\bar{1}4$ v.w.
122 w.	$\bar{1}22$ w.	422 v.w.	$4\bar{2}2$ v.w.
123 w.	...	423 w.	...
131 v.w.	131 w.	432 v.w.	...
132 v.w.	$\bar{1}32$ v.w.	511 w.	$5\bar{1}1$ w.
211 m.s.	$\bar{2}11$ m.s.	512 w.	$5\bar{1}2$ w.m.
212 v.w.	$\bar{2}12$ v.w.	513 w.m.	$5\bar{1}3$ w.
213 w.m.	213 w.	...	$5\bar{1}4$ w.
...	214 v.w.	...	$5\bar{2}2$ v.w.
...	$\bar{2}21$ w.	523 w.	$5\bar{2}3$ w.
222 v.w.	$\bar{2}22$ v.w.	611 w.	611 w.
231 v.w.	$\bar{2}31$ v.w.	...	$6\bar{1}2$ w.
311 v.w.	$\bar{3}11$ v.w.	...	$6\bar{1}4$ v.w.
312 v.w.	$\bar{3}12$ v.w.	621 v.w.	$6\bar{2}1$ w.
313 m.	313 w	622 w.	622 w.
321 v.w.	$\bar{3}21$ w.	623 w.	$6\bar{2}3$ w.
322 v.w.	$\bar{3}22$ v. w.	...	$7\bar{1}1$ v.w.
323 w.	$\bar{3}23$ w.	...	$7\bar{1}2$ w.m.
			$7\bar{1}3$ v.w.
			714 w.m.

The above tables shows that (hol) planes are halved when h is odd. Also (010) planes are halved. These halvings assign the crystal to the space group C_{2h}^5 with Γ *m* Bravais lattice. The number of molecules required by the space group is four. Since the number of molecules (mol. wt. 138·06) calculated from the dimensions of the unit cell and the specific gravity of the crystals (found to be 1·428) is also four (accurately 4·05), one *p*-nitroaniline molecule as a whole forms an asymmetric unit of the cell.

p-*Nitrotoluene*.

The crystals of *p*-nitrotoluene belong to the orthorhombic bipyramidal class and develop *b* (010), c (001), o (211), p (110) and q (011) faces. The axial ratio is

$$a : b : c = 0{\cdot}9111 : 1 : 1{\cdot}0971 \text{ (Groth, } \textit{loc. cit.}\text{, p. 362).}$$

These crystals have been studied by Sreenivasaiah (*Indian J. Phys.*, 1927, **2**, 151) by the Powder and Laue methods. He obtained the following values for the dimensions of the unit cell

$$a = 10{\cdot}17 \text{ Å}, \qquad b = 11{\cdot}18 \text{ Å}, \qquad c = 12{\cdot}3 \text{ Å}$$

and assigned the space group Q_h^1 to the crystal.

Pure substance crystallised from a mixture of alcohol and ether gave suitable single crystals for the present work. The substance vapourises very rapidly when exposed to the atmosphere, a crystal weighing about 1 mg. disappearing in nearly one hour. For this reason comparatively large crystals had to be used and shorter exposures were given.

The dimensions of the unit cell were calculated from the rotation photographs about the three principal axes and were found to be $a = 6{\cdot}41$ Å, $b = 14{\cdot}10$ Å, $c = 15{\cdot}39$ Å (Plates IV, V, VI)

The axial ratio is *a*: *b*: *c* = 0.4545: 1: 1·091 which agrees with that given in Groth except that *a* : *b* is halved.

Oscillation photographs were taken about the three crystallographic axes and the planes given in Tables III and IV identified.

TABLE III.

Axial planes.	Prism planes (okl).	Prism planes (hol)	Prism planes (hko).
002 v.s.	012 w.	105 w.m.	120 s
004 m.s.	014 w.	106 w.m.	130 m.s.
006 w.	016 w.	107 w.m.	140 s.
008 v.w.	018 or 028 w.	108 w.	150 w.m.
020 m.s.		201 m.	160 w.
040 v. s.	022 w.	202 w.	170 w.m.
050 w.m.	024 m.s.	203 w.m.	210 m.s.
060 w.m.	026 w.	204 w.	220 v.w.
080 w.	032 w.m.	206 m.	230 w.
200 s.	034 w.	207 w.m.	240 w.m.
	036 w.	208 w.	260 w.
	042 m.s.	301 w.	310 w.m.
	044 m.		330 w.
	046 v.w.		340 w.
	052 v.w.		
	054 w.		
	056 v.w.		
	062 v.w.		
	064 m.		

TABLE IV.

General planes.

111	s.	153	v.w.	235	w.m.
112	v.s.	154	v.w.	236	w.
114	w.m.	155	w.	241	w.m.
115	m.	156	v.w.	242	w.
116	w.	161	w.	243	m.
117	w.m.	163	w.	245	v.w
118	w.	164	v.w.	251	v.w.
121	m.s.	165	v.w.	253	v.w.
122	m.s.	171	w.m.	256	v.w.
123	m.s.	172	w.m.	261	w.
124	m.s.	173	w.	262	w.
125	w.m.	211	m.	263	v.w.
126	m.	213	w.m.	271 or 272	w.
127	m.s.	214	w m.		
128	v.w.	215	w.m.		
131	s.	216	m.	311	w.m.
132	s.	217	w.	312	v.w.
134	w.m.	218	w.	313	v.w.
135	w.	221	w.m.	314	w.
136	w.m.	222	w.	321 or 311	m.
137	v.w.	223	w.		
141	m.s.	224 or 214	w.m.	322	w.
142	m.			323	w.
143	m.			325	w.
144	v.w.	225	w.	331	w.m.
146	w.m.	226	w.	332	v.w.
151	w.m.	227	w.	333	v.w.
152	w.	231	w.	341 or 342	v.w.
		234	w.		

It will be seen from the above tables that (okl) planes are halved when l is odd. The crystals of *p*-nitrotoluene, therefore, belong to the space group C_h^5 which requires eight asymmetric units to complete the symmetry of the unit cell. The number of molecules calculated from the dimensions of the cell and the specific gravity of the crystals is also eight (accurately 7·98). The molecules in the cell are, therefore, asymmetric.

Sreenivasaiah's results calculated from the above expression are in close agreement with those obtained by us. The space group assigned by Sreenivasaiah (*loc. cit.*) is, however, not supported by the results of our investigation.

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Rotation photographs of p-*nitroaniline.*

Plate I.
a-axis

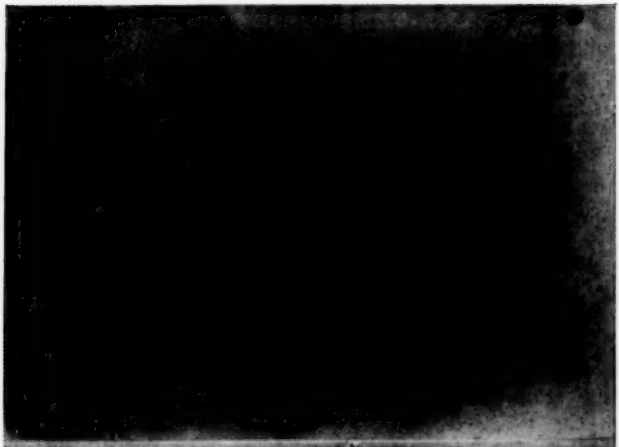
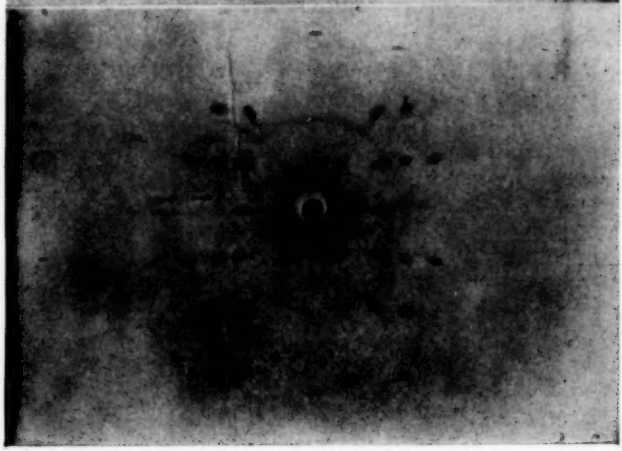


Plate II.
b-axis

Plate III.
c-axis



Rotation photographs of p-*nitrotoluene.*

Plate IV.
a-axis

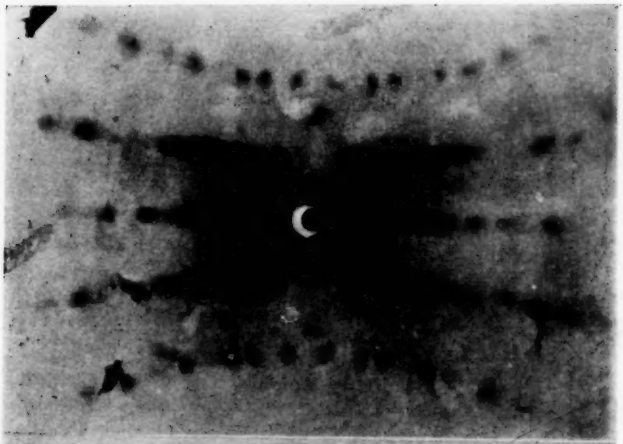


Plate V.
b-axis

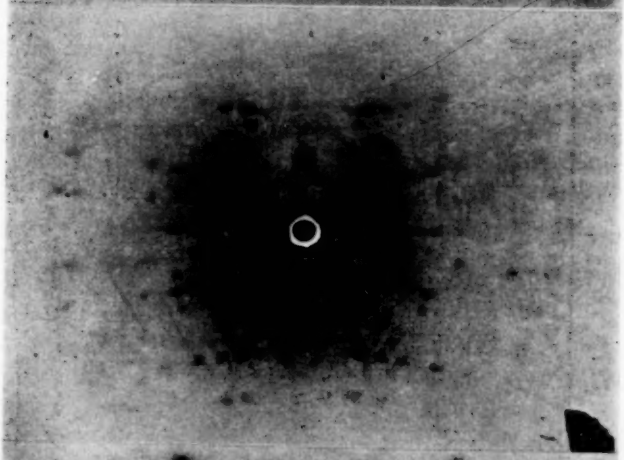
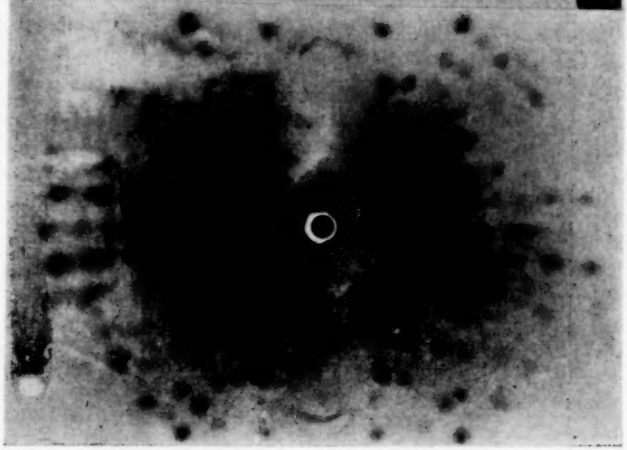


Plate VI.
c-axis



SPACE GROUP DETERMINATION OF THE CRYSTALS OF *ORTHO*- AND *PARA*- BENZTOLUIDES BY THE *X*-RAY ROTATING CRYSTAL METHOD.

By Mata Prasad and A. B. Khan.

Rotation photographs gave the following dimensions of the unit cell :—

p-benztoluide ($a = 26{\cdot}116$ A.U., $b = 9{\cdot}117$ A.U., $c = 9{\cdot}87$ A U.),

o-benztoluide ($a = 30{\cdot}54$ A.U., $b = 8{\cdot}24$ A·U., $c = 9{\cdot}246$ A.U).

Oscillation photographs taken about three principal axes at suitable small angles gave halvings corresponding to the space Q_h^5 for the first and Q_h^1 for the second.

1-o-*Benztoluide*.

o-Benztoluide crystallises from a mixture of ethylacetate and acetone in the rhombic class with the m{100}, a {100}, c{001} and r {101} faces. The axial ratio is $a : b : c = 1{\cdot}8449 : 1 : 0{\cdot}2810$ (*cf.* Groth, " Chemische Krystallographie," **V**, p. 165)

The substance was prepared by condensing benzoyl chloride with *o*-toluidine in an alkaline medium. The product was crystallised from methylated spirit. Suitable crystals were obtained by recrystallising the pure substance from a mixture of ethyl acetate and alcohol. The crystals were colourless, transparent and prismatic in shape. The melting point was found to be 143-44°. The substance was found to become dull on exposure to the atmosphere.

A Shearer gas-tube with copper target ($\lambda = 1{\cdot}54$) was used as the source of *X*-rays. Usually the m{110} faces had developed well on the crystals and the rotation photograph about the *c*-axis (Plate I) was easily obtained. The crystal was then suitably set so that the *a* and *b* axes were in turn coincident with the axis of rotation and the rotation photographs were taken (Plates II and III). On account of the unstability of the crystals rather short exposures were given.

The dimensions of the unit cell obtained from the rotation photographs are $a = 30{\cdot}54$ A.U., $b = 8{\cdot}24$ A.U., $c = 9{\cdot}246$ A.U.

The axial ratio agrees closely with that given in Groth (*loc. cit.*).

$$\left.\begin{array}{l} a : b : c :: 3{\cdot}707 : 1 : 1{\cdot}122 \\ \quad :: 2(1{\cdot}854) : 1 : 4\,(0{\cdot}2805) \end{array}\right\} \text{Author's}$$

$$:: 1{\cdot}8469 : 1 : 0{\cdot}2810 \qquad \text{Groth,}$$

except that $a : b$ is doubled and $c : b$ is quadrupled.

Oscillation photographs at suitable small angles were taken about all the three crystallographic axes and the spots indexed with the help of Bernal's charts. The planes identified have been collected and classified under different heads in Tables I and II.

TABLE I.

Axial planes.		Prism planes.					
Planes.	Intensity.	hol.	Intensity.	okl.	Intensity.	hko.	Intensity.
002	v.s.	102	m.s.	012	v.w.	210	v.s.
004	w.m.	104	v.m.	013	v.w.	220	m.
020	v.w.	202	m.s.	014	v.w.	230	v.w.
200	m.	204	m.	021	v.s.	410	s.
400	v.s.	302	s.	022	w.	420	w.
600	s.	402	s.	032	v.w.	430	v.w.
800	w.	404	m.	033	w.	610	v.s.
(10)00	m.s.	502	s.	041	w.m.	620	m.
(12)00	w.	504	m.			630	w.
(14)00	v.w.	602	v.s.			810	v.s.
		604	w.m.			820	m.s.
		702	v.s.			(10)10	w.
		704	m.			(10)20	w.
		802	w.			(10)30	w.m.
		902	w.			(12)10	v.w.
		(10)02	v.w.			(14)10	v.w.
		(10)04	m.			(14)20	v.w.
		(11)02	m.				
		(12)02	w.				
		(12)04	v.w.				
		(13)02	m.s.				
		(13)04	m.				

Table II.

General planes.

Planes.	Intensity.	Planes	Intensity.	Planes.	Intensity.	Planes.	Intensity.
111	m.s.	322	m.s.	612	m.	921	v.w.
112	m.	332	v.w.	613	m.	922	v.w.
113	v.w.	333	w.	614	m.s.	923	w.
121	m.s.	341	v.w.	622	m.	932	v.w.
122	m.	342	v.w.	623	v.s.	(10)11	w.m.
123	v.w.	411	w.	631	w.m.	(10)12	w.m.
131	v.w.	412	m.	711	w.m.	(10)13	w.m.
132	v.w.	413	m.	713	v.w.	(10)21	m.
133	w.	414	w.	721	m.s.	(10)22	v.w.
211	m.s.	421	s.	722	m.	(10)23	v.w.
212	m.s.	422	m.	731	m.s.	(10)32	v.w.
213	w.	432	v.w.	732	v.w.	(11)11	m.
214	w.	433	v.w.	733	v.w.	(11)12	w.m.
221	m.	511	v.s.	811	m.	(11)13	v.w.
222	m.	512	m.	812	v.w.	(11)14	v.w.
231	w.m.	513	m.	813	v.w.	(11)22	w.
232	v.w.	521	s.	814	v.w.	(11)31	m.
241	v.w.	522	w.	823	w.	(12)11	w.
242	v.w.	523	v.w.	831	m.s.	(13)11	m.
311	v.s.	524	v.w.	833	w.	(13)12	m.
312	m.	531	w.	911	w.	(13)21	v.w.
313	w.	532	v.w.	912	v.w.	(15)12	v.w.
314	w.	542	v.w.	913	v.w.	(16)11	v.w.
321	m.s.	611	v.s.	914	v.w.		

The above tables show that (hol) planes are halved when l is odd and (hko) are halved when h is odd. The crystals, therefore, belong to the space group $Q^{1,1}_{6}$ of the orthorhombic bipyramidal class.

The number of asymmetric units required to complete the symmetry of the cell is eight. The number calculated from the dimensions of the cell and the specific gravity of the crystals (1·205, Groth) is 8·005 (very nearly eight). Single molecules of *o*-benztoluide, therefore, form the asymmetric units of the cell.

p-*Benztoluide.*

Crystals belong to the rhombic bipyramidal class and develop a{100}, l {410}, n {210}, o {111}, c {001} and s {302} faces. The axial ratio is

$a : b : c :: 2{\cdot}8067 : 1 : 1{\cdot}0815$ (Groth, *loc. cit.*, p. 166).

The substance was prepared in the same way as described in the previous section except that the *para-* instead of the *ortho*-toluidine was used. The pure substance melted at 157°.

Colourless, transparent and well developed crystals were obtained by the slow evaporation of the solution of the substance in a mixture of acetone and alcohol. The crystals which were prismatic along the *c*-axis were quite stable when exposed to the atmosphere and almost all the faces described in Groth were identified. Rotation photographs about the *a*, *b*, and the *c* axes gave the following dimensions (*vide* Plates IV, V and VI),

$$a = 26{\cdot}11 \text{ Å}, \quad b = 9{\cdot}12 \text{ Å}, \quad c = 9{\cdot}87 \text{ Å}.$$

The axial ratio is $a : b : c :: 2{\cdot}863 : 1 : 1{\cdot}082$ which agrees very well with that given in Groth. The following tables (III and IV) give the list of planes identified on the oscillation photographs about the three principal axes.

TABLE III.

Axial planes.				Prism planes.			
Plane.	Intensity.	hol.	Intensity.	okl.	Intensity.	hko.	Intensity
002	s.	102	s.	021	m.s.	210	m.s.
200	m.	202	m.	022	w.	220	w.m.
400	s.	204	w.m.	023	w.	230	w.
600	v.w.	302	m.s.	024	w.m.	410	m.
(10)00	m.	304	m.	041	m.	420	v.w.
(12)00	w.m.	402	m.s.	042	w.	430	w.m.
		404	m.	043	w.m.	440	w.
		502	m.s.			610	v s.
		504	w.m.			620	w.m.
		602	m.s.			640	w.
		604	m.			810	m.s.
		702	m.s.			820	m.s.

TABLE III (contd.).

Prism planes. hol.	Intensity.	hko.	Intensity.
704	m.	840	w.m.
802	w.m.	(10)20	w.m.
804	w.m.	(10)30	w.m.
902	w.m.	(12)10	w.
(10)04	w.	(12)20	w.
(12)02	v.w.	(14)10	v.w.
(13)02	w.m.	(14)20	v.w.

TABLE IV.

General planes.

Planes.	Intensity.	Planes.	Intensity.	Planes.	Intensity.
111	s.	342	w.m.	812	v.w.
113	w.m.	343	w.m.	813	w.
114	v.w.	412	m.	822	w.
121	m.	413	m.	823	w.
122	v.w.	414	w.	831	m.
123	w.m.	422	v.w.	833	v.w.
124	v.w.	424	w.	834	v.w.
133	w.m.	441	w.m.	841	v.w.
142	w.	443	w.	911	v.w.
211	w.m.	511	v.s.	912	w.
212	w.m.	512	w.	913	v.w.
213	m.	513	w.m.	921	v.w.
214	w.	521	m.s.	922	v.w.
221	m.	522	m.	923	v.w.
222	w.m.	524	v.w.	924	v.w.
224	w.	532	w.m.	931	w.
231	w.m.	534	w.	933	v.w.
232	w.	542	w.	941	v.
233	w.	543	w.	(10)11	w.m.

Planes.	Intensity.	Planes.	Intensity.	Planes.	Intensity.
241	w.	611	m.s.	(10)13	v.w.
242	w.	612	w.m.	(10)22	w.
243	w.	613	w.m.	(10)23	v.w.
251	v.w.	621	w.	(10)31	w.
311	s.	624	v.w.	(10)32	w.
312	m.s.	631	w.m.	(11)11	v.w.
313	w.m.	632	w.	(11)13	w.
314	w.m.	633	s.	(11)21	v.w.
321	m.s.	642	w.	(11)22	v.w.
322	w.	711	m.	(11)23	v.w.
324	v.w.	721	w.	(11)31	w.m.
331	w.	722	w.	(12)12	v.w.
332	w.	723	w.	(12)13	w.
333	w.	731	w.m.	(12)21	v.w.
334	v.w.	741	v.w.	(13)11	w.
341	w.	811	w.	(13)12	w.

It will be seen from the above tables that

(okl) planes are halved when k is odd :

(hko) ,, ,, ,, ,, h is odd ;

and (hol) ,, ,, ,, ,, l ,, ,, .

The halvings correspond to the space group Q_h^{15} with Γ_o Bravais lattice.

The number of molecules required by the space group is eight. The number calculated from the demensions of the cell and the specific gravity of the crystals (1·202) is also eight (accurately 8·06). This shows that molecules of *p*-benztoluide in the unit cell are asymmetric.

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Oscillation photographs of o-benztoluide.

Plate I.
a-axis

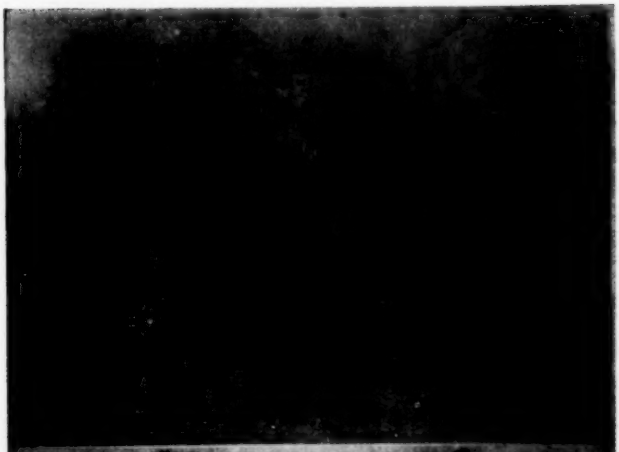


Plate II.
b-axis

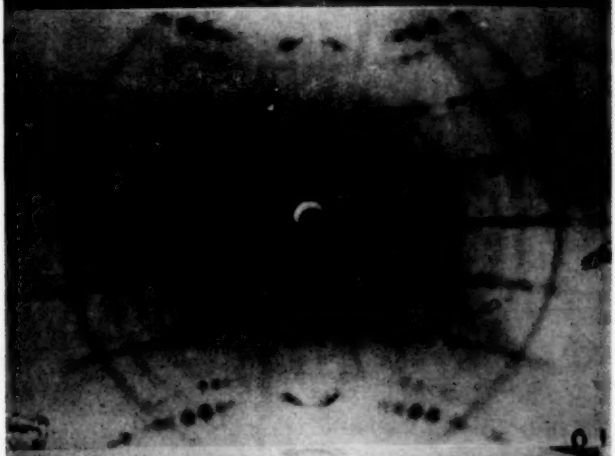
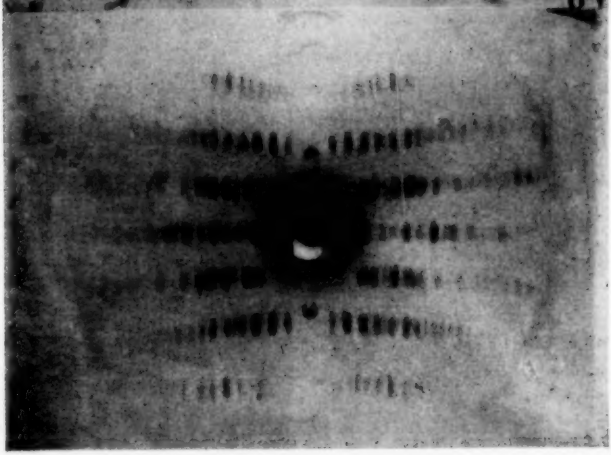


Plate III.
c-axis

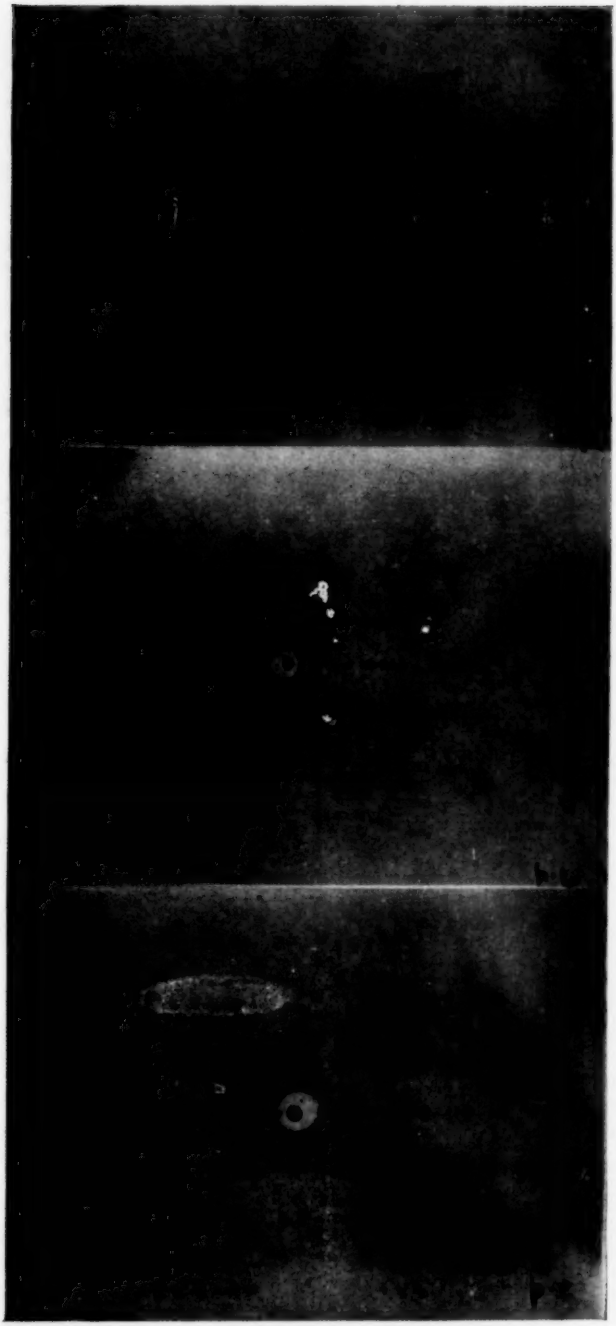


Oscillation photographs of p-*benztoluide.*

Plate IV.
a-axis

Plate V.
b-axis

Plate VI.
c-axis



ON THIOALDEHYDES AND THIOKETONES. PART II.

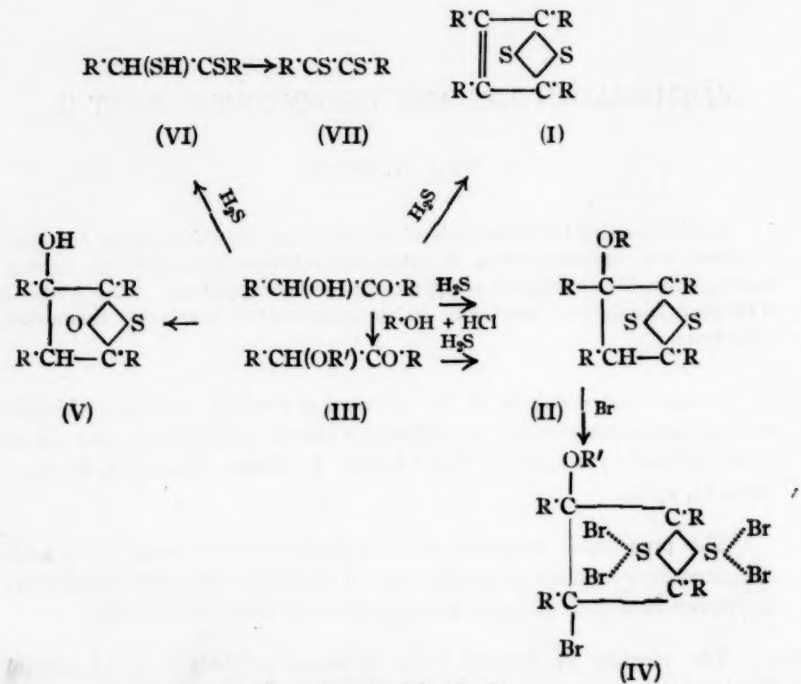
By S. K. Mitra.

A general method for the synthesis of thiophens and endothiothiophens has been described. The autocondensation of certain α keto-alcohols followed by an internal rearrrangement in an atmosphere of H_2S furnishes endothiothiophens. A new synthesis of thiophens has also been achieved by an application of this reaction to unsaturated γ-diketones.

Certain α-keto-alcohols in the presence of alcoholic hydrogen chloride undergo autocondensation to γ-diketones which subsequently give rise to furan and endoxyfuran derivatives (Irvine, *J. Chem. Soc.*, 1908, **93**, 950 ; 1907, **91**, 1386).

The pronounced tendency of thioketones to form cyclic compounds suggested the possibility of the synthesis of thiophen and endothiothiophen derivatives from such reactions in the presence of hydrogen sulphide.

The reaction of benzoin with hydrogen sulphide in ethyl alcohol furnished three compounds (A) m.p. 195°, (B) m.p. 126°, (C) m.p. 97°. The compound (A) does not possess any thiol or thioketonic group. It gives the thiophen reaction with isatin. It forms a tetrabromo derivative by absorbing four atoms of bromine, which loses all its bromine on heating. This fact suggests that all the bromine atoms are attached to the sulphur atoms. Structure (I) has been suggested for (A) since on reduction it gives tetraphenylthiophen. An attempted synthesis of (A) from the interaction of hydrogen sulphide and dibenzoylstilbene failed. The reaction afforded a quantity of tetraphenylfuran evidently from the reduction of dibenzoylstilbene by hydrogen sulphide. This fact affords an additional proof of the endoxyfuran structure of dibenzoylstilbene as suggested by Japp (*J. Chem. Soc.*, 1897, **71**, 1139). The presence of an ethoxy group has been confirmed in compound (B). Structure II (R′=Et) has been suggested for it since alcoholic hydrogen chloride could effect its conversion to (A). Evidence of a hydrogen atom in thiophen ring could also be gathered from its reaction with bromine. The substance absorbs five bromine atoms with evolution of hydrogen bromide and forms a compound for which structure (II) is evident.



(R = phenyl ; R′ = alkyl)

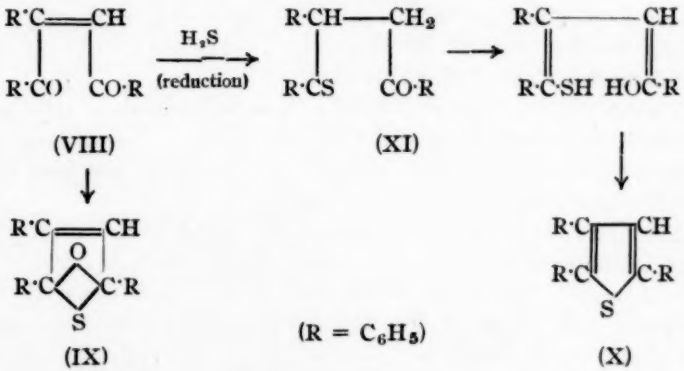
The position of the ethoxy group has also been confirmed by the synthesis of (B) from III (R′=Et) and further application of this reaction has been established by isolating the corresponding compound (R′=Me). Thus it is clear that two processes take place simultaneously in this reaction :

(*i*) Alkylation of benzoin in the presence of alcoholic hydrogen chloride (Fischer, *Ber.*, 1893, **26**, 2412).

(*ii*) Formation of a γ-thiodiketone which rearranges to endothiothiophen derivative like the corresponding oxygenated compounds (Irvine, *J. Chem. Soc.*, 1908, **93**, 952).

The existence of the thioketone group has been confirmed in (C) and the formation of benzil-diphenylhydrazone from its interaction with phenylhydrazine suggested the structure (VII) for it. Synthesis of this compound could not be effected since sulphur could not be introduced into benzil. The formation of this compound through oxidation of (VI) seems likely in analogy with the behaviour of benzoin under similar conditions (Irvine, *loc. cit.*). The reaction of

benzoin in methyl alcohol with hydrogen sulphide furnished along with compound (A) another substance (D). The compound (D) does not contain any ketonic, thioketonic or thiol group. It gives the thiophen reaction and can be reduced to thionessal. Structure (V) has been suggested for it. It is also noteworthy that (V) could not be acetylated. A similar case has been reported by Irvine (*loc. cit.*). The synthesis of endothiothiophens by the interaction of the corresponding diketone with hydrogen sulphide failed. Enolisation of the diketone followed by the elimination of water took place before sulphur was introduced. Under similar conditions unsaturated γ-diketone *e.g.* dibenzoylstyrene (VIII) gave an endoxythiophen (IX) and the corresponding thiophen (X). The formation of thiophen obviously necessitates the reduction of (VIII) to (XI) by hydrogen sulphide.



EXPERIMENTAL.

Tetraphenyl-2 : 5-endothiothiophen (I).—Hydrogen sulphide was passed (24 hours) through a solution of benzoin (15 g.) in alcoholic hydrochloric acid (500 c.c.) at 14°. The crystalline material was then collected and digested with ethyl alcohol. The insoluble portion was purified from a mixture of chloroform and ethyl alcohol and obtained as colourless silky needles, m.p. 195°. [Found : C, 79·7 ; H, 4·9 ; S, 14·8, M.W. (ebullioscopic in chloroform), 450. $C_{28}H_{20}S_2$ requires C, 80·0 ; H, 4·7, S, 15·2 per cent. M.W., 420].

The same compound was isolated by passing hydrogen sulphide through a solution of benzoin (5 g.) in glacial acetic acid (310 c. c.) saturated with hydrogen chloride and containing aqueous hydrochloric acid (29 c. c., *d* 1·19).

The substance gave (colouration) with isatin and concentrated sulphuric acid. It could not be reduced by zinc and hydrochloric acid and was slowly attacked by permanganate.

Tetraphenyl-2 : 5-endothiothiophen Tetrabromide.—The above mentioned thiophen (3 g.) was dissolved in chloroform (20 c.c.) containing bromine (8 g.). The green precipitate which was immediately thrown down furnished deep green needles from chloroform, m.p. 139°. The parent compound was regenerated by boiling with ethyl alcohol or acetic acid. (Found : Br, 43·1. $C_{28}H_{20}Br_4S_2$ requires Br, 43·2 per cent).

Action of Hydriodic Acid on (I) *and the Formation of Tetraphenylthiophen.*—Tetraphenylendothiothiophen (6 g.) was mixed with aqueous hydriodic acid (30 c.c., *d* 1·7) in a Zeisel flask, and heated to 127° (4 hours). The mixture after cooling was diluted with excess of water and the precipitate was extracted with excess of hot alcohol. The yellow material which separated from hot alcohol furnished thionessal, m.p. 184°, as colourless needles from glacial acetic acid. (Found : C, 86·7; H, 5·1. $C_{28}H_{20}S$ requires C, 86·6; H, 5·1 per cent).

Tretraphenyl - 3 - ethoxy- 4- hydro-2 : 5-endothiothiophen (II) *and Dithiobenzil* (VII).—Hydrogen sulphide was passed (2 hours) through a solution of benzoin (5 g.) in ethyl alcohol (100 c.c.) which had been previously saturated with dry hydrogen chloride at 0°.

The separated material was collected and boiled under reflux with a large excess of alcohol. The insoluble portion was tetraphenyl-2 : 5-endothiothiophen. The yellow material obtained from the alcoholic solution was purified from a mixture of acetone and alcohol and obtained as yellow cubes, m.p. 126°. [Found : C, 77·0 ; H, 5·6 ; S, 14·2 ; OEt, 8.9 ; M.W. (ebullioscopic in chloroform), 460. $C_{30}H_{26}OS_2$ (II) requires C, 77·2 ; H, 5·5 ; S, 13·7 ; OEt *, 9·6 per cent. M.W., 466]. The mother liquor of the reaction mixture was poured into ice and the semi-solid mass was collected and washed with hot aqueous alcohol. The residue furnished greenish yellow cubes from absolute alcohol, m.p. 97°. [Found : C, 69·2 ; H, 4·4; S, 26·0. $C_{14}H_{10}S_2$ (VII) requires C, 69·4; H, 4·1; S, 26·4 per cent].

The Formation of Tetraphenyl-2:5-endothiothiophen from Tetraphenyl-3-ethoxy-4-hydro-2:5-endothiothiophen.— Tetraphenyl-3-ethoxy-4-hydro-2:5-endothiothiophen (5 g.) was dissolved in alcoholic hydrochloric acid (100 c.c ,

* The estimation of ethoxyl in this compound was carried out by Zeisel's method with some modification. The exit gases from the flask were passed through water kept at 75°. The black precipitate in the flask containing silver nitrate, evidently a mixture of silver sulphide and iodide, was digested with concentrated nitric acid, filtered and washed with water. The silver iodide remaining was dried and weighed.

d 1·19) and the mixture was boiled for 6 hours. The solid separating on cooling was digested with absolute alcohol. The needles, m.p. 195°, isolated from chloroform were identical with tetraphenyl-2:5-endothiothiophen. The same compound was also isolated on treating the ethoxy thiophen (II) with aqueous hydriodic acid (*d* 1·7) at 90°.

Tetraphenyl-3-ethoxy-4-bromo-2:5-endothiothiophen Tetrabromide (IV).—Tetraphenyl-3-ethoxy-2:5-endothiothiophen (3 g.) was dissolved in chloroform (15 c.c.) to which bromine (6 g.) was subsequently added. The mixture was then kept at 15° (2 hours). The precipitate obtained by adding of an excess of chloroform melted at 178°. [Found : C, 41·3 ; H, 2·6 ; Br, 47·0. $C_{30}H_{23}OBr_5S_2$ requires C, 41·6 ; H, 2·8 ; Br, 46·3 per cent].

Action of Hydrogen Sulphide on Ethoxydeoxybenzoin (III, R′=Et) *and the Formation* of (I), (II) and (VII).—Hydrogen sulphide was passed through a solution (12 hours) of ethoxydeoxybenzoin (10 g.) in ethyl alcohol (100 c.c.) saturated with hydrogen chloride at 0°. The material which separated was boiled with ethyl alcohol in which (I) was insoluble. From the alcoholic solution (II) was isolated. This compound was also obtained by passing hydrogen sulphide through a solution of ethoxydeoxybenzoin (5 g.) in methyl alcohol (30 c.c.) saturated with dry hydrogen chloride. This fact proves that the solvent does not take part in this reaction. The mother liquor after isolation of (I) and (II) was rendered turbid with water. The precipitate furnished (VII) from alcohol.

3-Methoxy-4-hydrotetraphenyl-2:5-endothiothiophen (II, R = Me).—Methoxydeoxybenzoin (R=Me, 6 g.) was dissolved in methyl alcohol (100 c.c.) saturated with hydrogen chloride at 0°, and hydrogen sulphide was passed (12 hours). The precipitate was collected and extracted with boiling alcohol (200 c.c.). The residue was identified as tetraphenyl-2:5-endothiothiophen, m.p. 195°. The material obtained from the alcoholic extract furnished yellow needles from a mixture of acetone and alcohol. (Found : C, 77·0 ; H, 5·3 ; S, 13·7. $C_{29}H_{24}OS_2$ requires C, 77·0 ; H, 5·3 ; S, 14·1 per cent).

3-Hydroxy-4-hydrotetraphenyl-2:5-endoxythiophen (V).—Benzoin (20 g.) was dissolved in methyl alcohol (500 c.c.) saturated with hydrogen chloride. Hydrogen sulphide was passed (24 hours) at 15°. The mixture was poured into ice and the pasty mass was digested with acetone. The insoluble portion was tetraphenyl-2:5-endothiothiophen. The acetone solution furnished greenish yellow cubes, m.p. 165°, on addition of alcohol. (Found : C, 79·4 ; H, 5·4 ; S, 7·7. $C_{28}H_{22}O_2S$ requires C, 79·6 ; H, 5·2 ; S, 7·6 per cent).

The endoxythiophen (4 g.) was kept at 127° (4 hours) with aqueous hydriodic acid (30 c.c., *d* 1·7). Tetraphenylthiophen was isolated as before.

Action of Hydrogen Sulphide on Dibenzoylstilbene.—Hydrogen sulphide was passed (4 hours) through a solution of dibenzoylstilbene (3 g.) dissolved in alcohol (250 c.c.) saturated with hydrogen chloride. The precipitate furnished tetraphenylfuran, m.p. 174°, as colourless plates from acetic acid. (Found : C, 90·3 ; H, 5·2. $C_{28}H_{20}O$ requires C, 90·3 ; H, 5·3 per cent).

The Formation of 2:4:5-Phenylthiophen (X) *and its Endoxy-derivative* (IX).—Hydrogen sulphide was passed (6 hours) through a solution of dibenzoylstyrene (3 g.) in alcohol (50 c.c.) saturated with hydrogen chloride at 15°. The material which separated on standing overnight was boiled with excess of alcohol. The insoluble portion gave yellow plates (X) from acetic acid, m.p. 198°. (Found : C, 84·8 ; H, 5·3 ; S, 10·0. $C_{22}H_{16}S$ requires C, 84·6; H, 5·1; S, 10·2 per cent), and (IX) was isolated as yellow cubes from the alcoholic solution, m.p. 157°. (Found : C, 80·1; H, 4·8; S, 9·5. $C_{22}H_{16}OS$ requires C, 80·4; H, 4·8; S, 9·7 per cent).

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OPTICAL INACTIVITY OF GELATIN IN THE ADSORBED STATE AT THE LIQUID/LIQUID INTERFACE AND ITS USE AS A MEASURE OF ADSORPTION.

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Optical rotation for transparent emulsions of amyl acetate in gelatin-water-glycerol sols of various concentrations have been found experimentally and compared with those calculated from Beer's law for mixtures. The observed rotations are found to be less than the calculated from which it is inferred that the gelatin particles on being adsorbed on the oil globules become optically inactive.

The optical inactivity of adsorbed gelatin has been used as a measure of its adsorption at the liquid/liquid interface in transparent emulsions as well as such milky emulsions as could be made transparent by the addition of glycerine. From the difference between the calculated and observed values, the values of x/m (quantity of gelatin adsorbed by one g. of the oil) have been calculated and found to be nearly constant for the different quantities of the adsorbent used. The value of x/m increases with the increase in initial concentration.

It has been experimentally proved that the gelatin in the adsorbed state at the liquid/liquid interface loses optical activity only temporarily.

In emulsions the emulsifying agent is said to be adsorbed at the interface of the continuous and the disperse phases in accordance with the Gibbs-Thomson law, that is, it forms an envelope round each oil globule suspended in the dispersion medium.

There has long been a controversy over the protecting nature of this envelope; some take it to be a purely physical phenomenon, while others like Zsigmondy ("The Chemistry of Colloids", 1917, p. 112) and Langmuir (*J. Amer. Chem. Soc.*, 1917, **39**, 1845) postulate that chemical forces are acting. Bhatnagar and Shrivastava (*J. Phys. Chem.*, 1924, **28**, 730) have supported the presence of chemical forces between adsorbent and the adsorbed film by showing experimentally that various optically active sugars become inactive when adsorbed on colloidal particles of arsenious sulphide, antimony sulphide, etc. They have utilised this optical inactivity as a measure of adsorption of sugars on the collodial particles. Holmes and Chiid (*J. Amer. Chem. Soc.*, 1920, **42**, 2049), failed to determine quantitatively any adsorption of gelatin on oil globules in kerosene-in-water emulsions.

The object of the present investigation was to see if the gelatin being an optically active substance, becomes optically inactive on being adsorbed on oil globules in transparent emulsions of amyl acetate in gelatin-glycerol

sols of various concentrations, and whether this inactivity, being the difference between the calculated and observed optical rotations of the emulsions, could be utilised as a measure of its adsorption at the liquid/liquid interface to settle finally the cause of stability of the emulsions containing gelatin as the emulsifying agent.

EXPERIMENTAL.

It was found from preliminary experiments that transparent and very slightly chromatic emulsions could be prepared by dispersing amyl acetate in a mixture of gelatin-water sol of various concentrations and glycerine, each having a refractive index equal to that of the disperse phase. The gelatin-water sols were prepared as suggested by Sheppard and Houck (*J. Phys. Chem.*, 1930, **34**, 273). Pure gelatin (Merck's Gold Label) was used.

The proportions of various gelatin-water sols and glycerine in the various continuous phases used, and the optical properties of the latter are described in Table I. The refractive indices were determined by means of Abbe's refractometer whose prisms were enclosed in metal jackets through which water was run from a temperature regulator. The optical rotations were determined by means of half-shade polarimeter fitted with potassium dichromate gel filter and verniers reading to 0·01 degree. The liquid was enclosed in a 100 mm. tube, enclosed in a metal jacket, through which water was run from a temperature regulator; a 200-watt electric bulb was used as a source of light. The refractive index, n_D^{35} for amyl acetate was found to be 1·397, its optical rotation = 0 and its specific gravity ρ at the room temperature (35°) was found to be 0·8619.

TABLE I.

Cont. phase No.	Gelatin sol.	gel-water sol.	Glycerine.	%Gelatin in cont. phase.	n_D.	Rotation optical.	Rotation specific.
1	0·50%	56·50 c.c.	43·50 c.c	0·2825 g.	1·3970	−0·34	−120·4
2	0·75	56·75	43·25	0·4257	1·3970	−0·51	−119·8
3	1·00	57·00	43·00	0·5700	1·3970	−0·69	−121·1
4	1·50	57·50	42·50	0·8626	1.3970	−1·00	−116·0
5	2·00	58.00	42·00	1·1600	1·3970	−1·37	−118·1
6	2·50	58·50	41·50	1·4620	1·3970	−1·70	−116·2
7	3·00	59·00	41·00	1·7700	1·3970	−2·02	−114·1
8	3·50	59·50	40·50	2·0830	1·3970	−2·37	−113·8

The emulsions were prepared in 125 c.c. glass-stoppered bottles; 25 c.c. of the continuous phase were introduced in each bottle and the oil dispersed in it gradually by shaking in an electrically driven shaker, giving up and

down angular motion (similiar to shaking with hand) with 6″ stroke and 230 oscillations per minute.

After the required percentage content of the disprese phase was reached the emulsions were homogenised and freed from air-bubbles by placing the bottles in a water-bath at 45°. The clear emulsions were allowed to cool to room temperature before introducing them in the polarimeter observation tube. The optical rotations of the emulsions were found within 2 hours after preparation, as the lowest (28·57%) creamed after about 3 hours. The optical rotations for the emulsions were also calculated from the Beer's law for mixtures $B' = B/(n+1)$. This law was tested practically by adding equal quantity of glycerine to 1·16% gelatin-water sol. The optical rotation at 26° for 1·16% gelatin-water sol is −1·37 and the optical rotation at 26° for 1·16% gelatin-water sol + glycerine (1:1) is −0·68, half of the above as required by the law. Emulsions of each concentration of gelatin were prepared thrice and studied independently for the optical rotation. The readings varied within 0·02. The mean of tiie three different readings were taken as final. The observed and calculated optical rotation of emulsions prepared in gelatin sols of various concentrations and the values of x/m, calculated from the difference of the two, are shown in Table II.

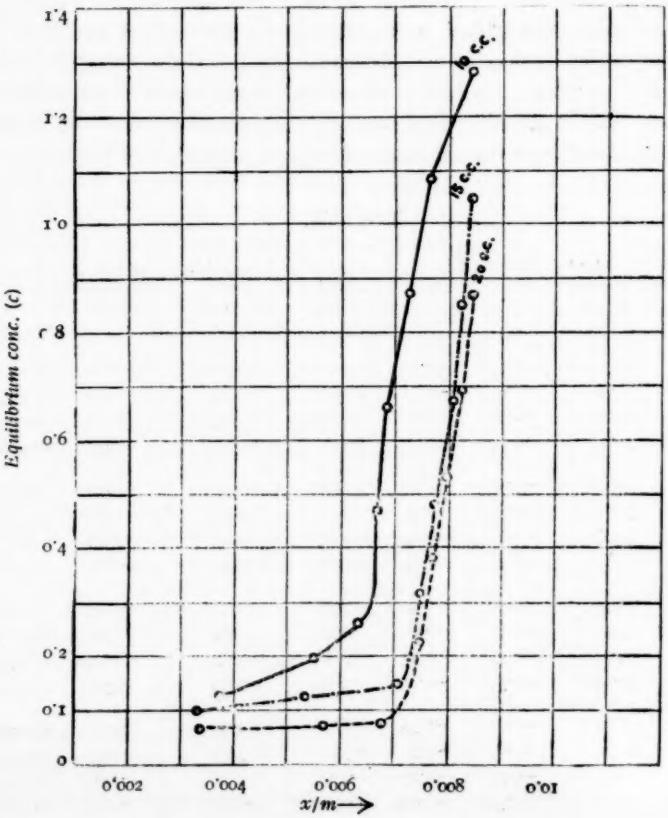
TABLE II.

Cont. phase No.	% content of disperse phase (v).	Rotation calc.	Rotation obs.	% of geiatin actual (d).	in emulsion calc. (c).	Gelatin adsorbed ($d-c$).	$\frac{x}{m}=\frac{d-c}{\rho v}$.
1	28·57 c.c.	−0·24	−0·16	0·1994 g.	0·1329 g.	0·0665 g.	0·000701
	37·50	−0·21	−0·12	0·1744	0·0997	0·0747	0·002311
	44·44	−0·19	−0·08	0·1579	0·0665	0·0914	0·002385
	50·00	−0·17	−0·03	0·1412	0·0249	0·1163	0·002699
2	28·57	−0·35	−0·23	0·3041	0·1920	0·1121	0·004552
	37·50	−0·32	−0·15	0·2661	0·1252	0·1409	0·004359
	44·44	−0·28	−0·09	0·2367	0·0751	0·1616	0·004709
	50·00	−0·26	−0·03	0·2128	0·0250	0·1878	0·004356
3	28·57	−0·48	−0·32	0·3967	0·2642	0·1325	0·005380
	37·50	−0·42	−0·18	0·3470	0·1487	0·1983	0.006135
	44·44	−0·37	−0·10	0·3058	0·0825	0·2233	0·005829
	50·00	−0·34	−0·05	0·2809	0·0413	0·2396	0·005559
4	28·57	−0·71	−0·55	0·6120	0·4741	0·1379	0·005601
	37·50	−0·62	−0·38	0·5388	0·3275	0·2113	0·006537
	44·44	−0·55	−0·27	0·4793	0·2328	0·2465	0·006434
	50·00	−0·50	−0·19	0·4310	0·1638	0·2672	0·006198

TABLE II (contd.).

5	28·57	−0·96	−0·79	0·8162	0·6688 g.	0·1474	0·005985
	37·50	−0·84	−0·58	0·7137	0·4910	0·2227	0·006892
	44·44	−0·75	−0·45	0·6351	0·3810	0·2541	0·006633
	50·00	−0·67	−0·34	0·5715	0·2878	0·2837	0·006583
6	28·57	−1·20	−1·02	1·0330	0·8778	0·1552	0·006302
	37·50	−1·05	−0·78	0·9036	0·6713	0·2323	0·007188
	44·44	−0·93	−0·62	0·8004	0·5335	0·2669	0·006968
	50·00	−0·84	−0·49	0·7230	0·4217	0·3013	0·006990
7	28·57	−1·43	−1.24	1·2510	1·0860	0·1650	0·006701
	37·50	−1·25	−0·98	1·0950	0·8588	0·2362	0·007305
	44·44	−1·11	−0·78	0·9727	0·6923	0·2804	0·007319
	50·00	−1·00	−0·64	0·8764	0·5610	0·3154	0·007318
8	28·57	−1·68	−1·47	1·4760	1·2910	0·1850	0·007513
	37·50	−1·47	−1·19	1·2910	1.0460	0·2450	0·007580
	44·44	−1·30	−0·98	1·1470	0·8610	0·2860	0·007466
	50·00	−1·17	−0·81	1·0320	0·7117	0·3203	0·007430

FIG. 1.



The graphs showing the relation between the equilibrium concentration c (calculated from the observed rotations) and x/m, the quantity adsorbed by 1 g. of the disperse phase, are shown in Fig. 1.

Since it was found that the values of x/m were nearly constant with different quantities of the adsorbent by the above mentioned method, it was worth while to apply the same for measuring the adsorption of gelatin in milky emulsions of amyl acetate in gelatin-water sol.

The various quantities (10, 15, 20, 25 c.c.) of amyl acetate were added to 14·25 c.c. of 10% gelatin-water sol and four milky emulsions were obtained by first shaking with hand after every addition of 2 c.c. and then in the mechanical shaker. It was not possible to prepare these emulsions by directly shaking in the mechanical shaker, as the disperse phase separated immediately after the shaking was stopped.

To each of these milky emulsions, 10·75 c.c. of glycerine (dehydrated and having refractive index $n_D^{26} = 1{\cdot}4700$) were added to make it transparent. After addition of glycerine, it was once more shaken in the mechanical shaker, and then homogenised, and freed from air-bubbles.

The observed optical rotations and the values of c, x, and x/m, calculated therefrom, are shown in Table III.

TABLE III.

Vol. of disperse phase.	Rotation calc.	Rotation obs.	% of gelatin actual d.	% of gelatin calc c.	Adsorbed gelatin $(d-c)=x$.	$\frac{x}{m}$.
28·57 c.c.	−0·48	−0·42	0·3967 g.	0·3468 g.	0·0499 g.	0·002026 g.
37·50	−0·42	−0·33	0·3470	0·2726	0·0744	0·002302
44·44	−0·37	−0·26	0·3058	0·2147	0·0911	0·002378
50·00	−0·34	−0·21	0·2809	0·1734	0·1075	0·002494

On comparing the results of Table III with those of emulsion No. 3 of Table I, it was observed that adsorption of gelatin from 1% gelatin-water sol was less than from 0·57% gelatin-water-glycerol sol. This was found to be due to the lower interfacial tension between the two phases in the latter case than in the former. As determined by the drop-pipette method, the interfacial tension in the former case was 8·134 and in the latter was 7·3940 dynes/cm. It is concluded from the above results that the quantity of gelatin adsorbed from 1% gelatin-water sol remains unaffected on addition of glycerine.

In order to investigate whether the gelatin in the adsorbed state becomes temporarily or permanently optically inactive, the stable 50% emulsions of amyl acetate in various gelatin-water-glycerol sols, were partially broken mechanically by adding 10 c.c. of amyl acetate (*i.e.* by overloading) and shaking the same in the mechanical shaker continuously for one hour, as suggested by Stirling (*J Inst. Petrol. Tech.*, 1920, **6**, 382). The oil, thus separated, was measured and the optical rotation of the remaining emulsion was found out as shown in Table IV.

TABLE IV.

% Conc. of gelatin in cont. phase.	Amyl acetate separated (including extra 10 c.c.).	% Content of amyl acetate in the remaining emulsion.	Optical rotation of the remaining emulsion
0·2825 g.	35·00 c.c.	0·0c	−0·34
0·4257	34·50	1·95	−0·46
0·5700	32·00	10·71	−0·48
0·8626	29·50	14·75	−0·67
1·1600	26·00	26·50	−0·82
1·4620	25·00	28·57	−1·02
1·7700	24·50	29·58	−1·19
2·0830	24·10	30·36	−1·38

On comparing the optical rotations of the remaining emulsions with those of the corresponding 50% emulsions, it is observed that in all cases, there is an increase in the optical rotation. In the case of 0·2825% gelatin sol, all the disperse phase is separated and the continuous phase remains behind ; the optical rotation of this continuous phase is exactly the same as the original (compare Tables I & IV), *i.e.*, the gelatin in the original state has been recovered. Similarly in the case of 1·4620% gelatin sol, 28·57% emulsion remains behind ; the optical rotation of this emulsion is the same as that given in Table II. From these experimental facts it is concluded that the gelatin films, optically inactive, become active on breaking. So there is a temporary loss of optical activity by gelatin particles adsorbed on the oil globules.

DISCUSSION.

It is concluded from Table II that gelatin becomes optically inactive in the adsorbed state because the observed value of the optical rotation for a transparent emulsion is less than that calculated from Beer's law. This agrees well with the observation made by Bhatnagar and Shrivastava (*loc. cit.*) that sugars become optically inactive when adsorbed on colloidal particles. It is also observed that more and more quantity of gelatin is adsorbed as the quantity of the adsorbent (disperse phase) is increased.

Holmes and Child (*loc. cit.*) found out experimentally by creaming method that no gelatin is adsorbed on the oil globules and hence there are no protecting films round them. They attributed the stability of gelatin emulsions to the viscosity of the continuous phase. Narwani (*Kolloid Z.*, 1935, **70**, 297) concluded from the study of the surface tension, viscosity, and ageing effect of emulsions of cod-liver oil in gelatin-glycerol sols, that gelatin films exist round the oil globules. The present work confirms quantitatively the adsorption of gelatin at the liquid/liquid interface and thus settles the long standing dispute over the existence of gelatin films round the oil globules.

It was not possible to prepare emulsions of higher percentage content of the oil than 50% by using continuous phases, containing less than 0·5% of gelatin. This fact is also confirmed by a very low optical rotation obtained from 50% emulsions, prepared in gelatin sols of lower concentrations.

The value of x/m (quantity of gelatin adsorbed by 1 g. of disperse phase) in case of various percentage contents of the disperse phase of an emulsion are fairly constant. The value of x/m goes on increasing with the increase in the initial concentration of gelatin. The curve showing the relation between c, the equilibrium concentration and x/m resembles that obtained by Briggs (*J. Phys. Chem.*, 1915, **19**, 210) for emulsions of benzene in sodium oleate solutions. He titrated 10 c.c. emulsion directly with $N/20$-HCl, assuming that the adsorbed sodium oleate did not react with HCl. Hence our assumption that the gelatin becomes optically inactive on being adsorbed on oil globules, seems to hold good.

This loss of optical activity may be attributed either to orientation of gelatin molecules at the interface as suggested by Langmuir (*J. Amer. Chem. Soc.*, 1917, **39**, 354, 541) or to catalytic condensation of the closely packed, specifically strained, and orientated protein molucules into a single vast chemical aggregate—activated amino- and carboxyl groups of neighbouring molecules uniting by peptide linkages with formation of water as suggested by Ramsden (*Trans. Faraday Soc.*, 1926, **22**, 484). But since the optically inactive gelatin film again becomes active as concluded from the results shown in Table IV, the temporary orientation of the adsorbed molecules is more likely than their chemical condensation.

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HALOGENATION. PART XX. HALOGENATION OF FLUORENE.

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The following halogen compounds of fluorene have been prepared and the position of the halogen atoms with the exception of one or two definitely established :—2-bromo-7-chlorofluorene, 2-chloro-7-iodofluorene, 2-iodo-7-chlorofluorene, 2-bromo-7-iodofluorene, 2-iodo-7-bromofluorene, 2:7-dibromo-6 (?)-iodofluorene, 2:7-dichloro-6 (?)-bromofluorene, 2 :7-diiodofluorene.

From the study of the literature, it appears that simple halogen derivatives alone of fluorene have been obtained before. Of the chloro derivatives of fluorene, mono-, di-, tri-, and penta-chloro derivatives have been prepared by the direct action of chlorine under different conditions. By the action of bromine under the varying conditions of the experiments, similar bromo derivatives have been obtained. Of the iodo derivatives, only two mono-iodo derivatives, 2-iodo-, and 9-iodofluorene have been prepared (Chanussot, *Anal. Assoc. Quim.* Argentina, 1927,**25**, 5 ; Wanscheidt, *Ber.*, 1926, **59**, 2092). No attempt has been made before for the preparation of mixed halogen derivatives of fluorene. The authors have attempted to prepare such compounds as a result of which the following mixed halogen compounds have now been obtained. 2-chloro-7-bromofluorene, 2-bromo-7-chlorofluorene, 2-chloro-7-iodofluorene, 2-iodo-7-chlorofluorene, 2-bromo-7-iodofluorene, 2-iodo-7-bromofluorene, 2 :7-dibromo-6(?)-iodofluorene, 2:7-dichloro-6(?) -bromofluorene.

Most of the above compounds have been obtained by more than one method so that it has been possible to ascertain with certainty the position of the halogen atoms in the fluorene molecule. For example, 2-chloro-7-bromofluorene has been prepared by brominating 2-chlorofluorene and also by replacing the amino group in 2-chloro-7-aminofluorene by bromine.

In addition to the above compounds 2:7-diiodofluorene has been prepared for the first time by the direct iodination of fluorene in glacial acetic acid at 100° using a mixture of concentrated nitric acid and fuming sulphuric acid and also by replacing the amino group or groups in 2:7-

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diaminofluorene or in 2-iodo-7-aminofluorene by iodine. Hence the positions of the iodine atoms in the molecule of this compound could be known with certainty.

EXPERIMENTAL.

2 - Chloro - 7 - bromofluorene. (*i*) *From 2- Chlorofluorene.*—2-Chlorofluorene (5 g.), prepared by the direct action of chlorine on fluorene in benzene at 80°-90° in presence of iodine (Buffle, *Helv. Chim. Acta*, 1932, **15**, 1483), was dissolved in chloroform (30 c.c.) and iron (0.5 g.) was introduced as a halogen carrier. Keeping the temperature of the solution below 10°, liquid bromine (1·5 c.c.) was added drop by drop in 25 minutes. The resulting product was gently warmed on a water-bath to drive out the hydrogen bromide, filtered from iron and allowed to stand for an hour for crystallisation. The solid separating was recrystallised twice from carbon disulphide when colourless, transparent, monoclinic crystals were obtained, m. p. 157°, yield 3.4 g. It is very soluble in carbon disulphide and chloroform, and sparingly in benzene and alcohol. (Found : Total halogen, 40.84. $C_{13}H_8ClBr$ requires Total halogen, 41·32 per cent).

(*ii*) *From 2-Chloro-7-aminofluorene.*—A solution of 2-chloro-7-aminofluorene (5·g.), prepared by nitrating 2-chlorofluorene and reducing the chloronitro compound (Courtot and Vignati, *Compt. rend.*, 1927, **184**, 1779), was diazotized by sodium nitrite (2 g.) solution in 5% aqueous hydrochloric acid at 5°, in the usual manner. This mixture was slowly added to cuprous bromide (3·5 g.) dissolved in hydrobromic acid (50 c.c.) and shaken, when a greenish yellow product separated out. The product was allowed to stand overnight, filtered, washed with water and then crystallised from glacial acetic acid as colourless crystals, m. p. 157°, yield 2·9 g. It is identical with the compound obtained in the preceding experiment.

2- Bromo-7-chlorofluorene. (*i*) *From 2-Bromofluorene.*—Through a solution of 2-bromofluorene (Hodkinson and Mathews, *J. Chem. Soc.*, 1883, **43**, 163) in chloroform (25 c.c.), a current of dry chlorine was passed in the presence of iodine (0·5 g.) in diffused day light for an hour. The chloroform was allowed to evaporate and the solid residue washed with aqueous sodium hydroxide (5%) until free from iodine, then with water. It was crystallised twice from glacial acetic acid as transparent needles, m.p. 156-57°, yield 3·7 g. It is soluble in acetone, carbon tetrachloride and carbon disulphide. (Found : Total halogen, 40·56. $C_{13}H_8ClBr$ requires Total halogen, 41·32 per cent).

(*ii*) *From 2-Bromo-7-aminofluorene.*—2-Bromo-7-aminofluorene (5g.) (Courtot and Vignati, *loc. cit.*) was dissolved in 5% hydrochloric acid and diazotised with sodium nitrite (1·8 g.) in the usual manner. Copper powder (2 g.) was added and the mixture allowed to stand for about 20 hours in cold water. The product was extracted with benzene, washed with water, dried over calcium chloride and allowed to stand and the solid separating was crystallised from carbon disulphide, m.p. 155·5°, yield 0·7 g. The properties agree with the compound prepared by the preceding method.

2-Chloro-7-iodofluorene. (*i*) *From 2-Chlorofluorene.*—A mixture of concentrated nitric acid (3 c.c.) and fuming sulphuric acid (2 c.c.) was added in drops to 2-chlorofluorene, (5 g.) iodine (4·5 g.) and glacial acetic acid (25 c.c.) in about 20 minutes and the whole refluxed for about 3½ hours on a water-bath. The solution was cooled, poured into water and the solid separating extracted with benzene, the benzene extract washed with aqueous sodium hydroxide (5%) and then with water, dried over fused calcium chloride, allowed to stand, when crystals separated, yield 4·1 g. On crystallisation from warm acetic acid it melts at 121·5°. It is soluble in carbon tetrachloride, acetone and ether. (Found : Total halogen, 49·13. $C_{13}H_8ClI$ requires Total halogen, 49·77 per cent).

(*ii*) *From 2-Chloro-7-aminofluorene.*—Diazotised 2-chloro-7-aminofluorene (5 g.) was treated with a solution of potassium iodide (4·9 g.) in water (20 c.c.) at about 8°. The mixture was heated on a water-bath for 1 hour, extracted with benzene. The extract was washed with aqueous sodium hydroxide (5%) and then with water, dried over calcium chloride and allowed to stand when pale yellow crystals separated, m.p. 121-22° after crystallisation from glacial acetic acid, yield 1·9 g. It is soluble in carbon disulphide and chloroform.

2-Iodo-7-chlorofluorene.—2-Iodo-7-nitrofluorene (Chanussot, *loc. cit.* was reduced with zinc dust and aqueous alcohol (30 c.c. of alcohol and 10 c. c. water) and dissolved in aqueous hydrochloric acid (5 c. c. of *d* 1·17 and 20 c. c. of water) and treated with sodium nitrite (2 g. in 15 c.c. of water) solution below 10°. Cuprous chloride (3 g.), dissolved in dilute hydrochloric acid (25 c.c.), was then care fully added and the mixture allowed to stand for 16 hours. The solid separating was washed with water, extracted with benzene, and the extract allowed to stand. After repeated crystallisation from hot glacial acetic acid pale yellow needles, m.p. 122°, were obtained, yield 2·1 g. It is freely soluble in chloroform and acetone and end moderately in alcohol. (Found: Total halogen, 48·93. $C_{13}H_8ClI$ requires Total hologen, 49·77 per cent).

2-Bromo-7-iodofluorene. (*i*) *From 2-Bromofluorene.*—A mixture of 2-bromofluorene (5 g.), iodine (3·4 g.) and glacial acetic acid (20 c.c.) was treated with a mixture of fuming sulphuric acid (2 c. c.) and concentrated nitric acid (3 c. c.) and the whole refluxed on a water-bath for 4 hours. The solid obtained on pouring the cold mixture into water was extracted with benzene. The benzene extract was washed with dilute sodium hydroxide (5%) solution, and then with water, dried over calcium chloride and allowed to stand. The solid separating was crystallised from hot acetic acid as yellowish needles, m.p. 162°, yield 3·7 g. It is soluble in benzene and chloroform. (Found : Total halogen, 57·17. $C_{13}H_8BrI$ requires Total halogen, 55·80 per cent).

(*ii*) *From 2-Bromo-7-aminofluorene.*—2-Bromo-7-aminofluorene (5 g.) was diazotised and treated with potassium iodide (2%) solution and allowed to stand overnight. It was then extracted with carbon tetrachloride and the extract washed with aqueous sodium hydroxide (5%) solution and with water dried over calcium chloride and allowed to stand. The solid separating was crystallised from glacial acetic acid, m. p. 161-62°, yield 2·2 g. It is found to be identical with the compound obtained by the direct iodination of 2-bromofluorene.

2-Iodo-7-bromofluorene.—2-Iodo-7-aminofluorene (5 g.) was diazotised and treated with cuprous bromide (3·5 g.) in aqueous hydrobromic acid (20 c. c.) and left to stand overnight in the cold. A pale yellow solid separated which was filtered and crystallised in needles from acetic acid, m. p. 160·5°, yield 2·3 g. (Found : Total halogen, 54·31. $C_{13}H_8BrI$ requires Total hologen, 55·80 per cent).

2:7-Dibromo-6(?)-iodofluorene.—2:7-Dibromofluorene (5 g.), prepared by the action of bromine on fluorene in carbon disulphide (Fittig and Schmitz, *Annalen*, 1878, **193**, 137; Werner and Egger, *Ber.*, 1904, **37**, 3029), was heated with iodine (2·6 g.) and glacial acetic acid (20 c. c.) and heated under reflux on a water-bath and a mixture of concentrated nitric acid (3 c.c.) and fuming sulphuric acid (2 c.c.) was added 5 drops at a time, and the heating continued for about $3\frac{1}{2}$ hours. When cooled, the mixture was poured into water, filtered and the solid washed several times with aqueous sodium hydroxide (5%) and finally with water. It was extracted with chloroform, the chloroform extract dried over calcium chloride and left to stand. It was crystallised from acetic acid as yellowish granular particles, m. p. 146·5°, yield 4·1 g. It is soluble in carbon disulphide, acetone and benzene. (Found: Total halogen, 65·27. $C_{13}H_7Br_2I$ requires Tolal halogen, 63·78 per cent).

2:7-Dichloro-6 (?)-bromofluorene.—2:7-Dichlorofluorene (5 g.), prepared by passing chlorine through a solution of fluorene in chloroform (Hodkinson

and Mathews, *loc. cit.*) was dissolved in chloroform (25 c. c.) and iron (0·5 g.) was introduced as a halogen carrier. Liquid bromine (2 c.c.) was added drop by drop in course of 20 minutes. Some crystals separated out which were filtered, washed with carbon tetrachloride and filtered free from iron. The carbon tetrachloride extract gave needles, m. p. 143°. yield 3·5 g. It is soluble in acetone, benzene and carbon disulphide. (Found: Total halogen, 46·71. $C_{13}H_7Cl_2Br$ requires Total halogen, 48·08 per cent)

2 : 7-*Diiodofluorene*. (*i*) *From* 2:7-*Diaminofluorene*.—A solution of 2 : 7-diaminofluorene (Schmidt and Hindever, *Ber.*, 1931, **64**, 1793) in dilute hydrochloric acid was diazotised at 5° with sodium nitrite (2 g.) solution and then treated with potassium iodide solution (4 g. in 20 c.c. of water) and allowed to stand overnight. It was extracted with benzene, washed first with aqueous sodium hydroxide, then with water, decolourised by animal charcoal, filtered, dried and allowed to stand. On recrystallisation from glacial acetic acid pale brown needles, m.p. 155·5°, soluble in chloroform, ether, acetone, and warm alcohol, were obtained, yield 2·8 g. (Found : I, 59·85. $C_{13}H_8I_2$ requires I, 60·90 per cent).

(*ii*) *From* 2-*Iodo*-7-*aminofluorene*.—2-Iodo-7-aminofluorene (5 g.) was diazotised and treated with potassium iodide (2·5 g.) solution and treated as mentioned above, when crystals of diiodofluorene were obtained, yield 2·9 g.

(*iii*) *From Fluorene*.—Fluorene (5 g.) iodine (8 g.) and glacial acetic acid (25 c.c.) were heated on a water-bath and a mixture of strong nitric acid (3 c.c.) and fuming sulphuric acid (2 c.c.) was added, a few drops at a time in half an hour, and the whole refluxed for about 4 hours. On cooling, the solution was washed with water, extracted with benzene and the benzene extract washed first with dilute sodium hydroxide solution and then with water. It was dried over calcium chloride and allowed to stand and the crystals were purified by repeated crystallisation from alcohol, yield 1·9 g.

Work on similar lines is in progress.

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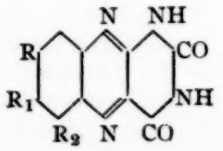
SYNTHESIS IN THE ALLOXAZINE, *ISO*ALLOXAZINE (FLAVIN) AND LUMAZINE GROUPS. PART II. SYNTHESIS OF SOME AMINO DERIVATIVES OF ALLOXAZINE AND THIOALLOXAZINE.

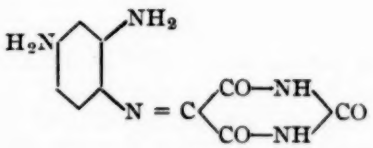
By K. Ganapati.

In attempting to explore new methods of synthesis of compounds of the alloxazine group with substituents in benzene ring, it has been found that 1 :2 :4-triaminobenzene condenses with violuric acid yielding an amino-alloxazine, similar to the condensation product of violuric acid with *m*-phenylenediamine. Thioviloluric acid, like violuric acid, condenses with *m*-phenylenediamine to yield an amino-2-thioalloxazine. These compounds are expected to be of use in some chemotherapeutic investigations.

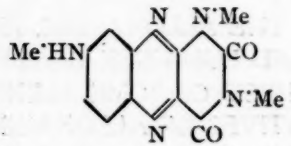
Since the synthesis of alloxazines with various substituents in the benzene ring by the method of Kuhn requires the corresponding substituted *ortho*-diamines, which are not easily accessible, it has now been tried to explore other possible methods for their synthesis. It has been reported by Kuhn and Cook (*Ber.*, 1937, **70**, 763) that the synthesis of alloxazine by the condensations of (*a*) *o*-aminophenol with uramil, (*b*) bromobarbituric acid with *o*-phenylenediamine, and (*c*) aniline with violuric acid was not successful. Piloty (*Annalen*, 1904, **333**, 44), however, condensed *m*-phenylenediamine with violuric acid and obtained a dyestuff which he called "ureidamidoazine." From its physical properties he regarded it to be an azine of the quinoxaline group but has refrained from suggesting a definite formula though he appears to favour its formulation as 7-aminoalloxazine (I, $R=NH_2$; $R_1=R_2=H$) also adopted in Beilstein's Handbuck (Bd. XXIV, p. 507; Bd. XXVI, p. 591). The British Chemical Abstracts (1904, *i*, 821) have, however, recorded the formula (II). In justification of the assignment of the alloxazine structure to this compound, it has now been found, besides other properties (*vide* experimental), that it yields with excess of diazomethane only a *trimethyl* derivative which can be represented by (III) on the basis of the formula (I) for the original compound.



(I)



(II)



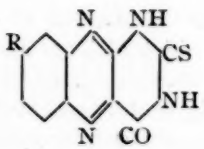
(III)

The condensation of violuric acid with *m*-phenylenediamine to yield an alloxazine can theoretically proceed in two directions to furnish either 7-aminoalloxazine or 5-aminoalloxazine (I. $R = R_1 = H$; $R_2 = NH_2$) according as the oximino group of violuric acid condenses in the *ortho*- or the *para*-position to the unattacked amino group in *m*-phenylenediamine. It is generally known that a substituent in the benzene ring (in the *meta* position to the group participating in the cyclisation) always activates the *para*-position in preference to the *ortho*; but in some particular cases the cyclisation has actually been shown to take place in the *ortho*-position (Späth and Kruta, *Monatsh.*, 1928, **50**, 341; *Ber.*, 1929, **62**, 1024; *Ann. Rep. Chem. Soc.*, 1929, **26**, 177). It appeared, therefore, to be of interest to condense 1:2:4-triaminobenzene hydrochloride with alloxan which can yield either 7-aminoalloxazine or 6-aminoalloxazine (I, $R_1 = NH_2$; $R = R_2 = H$) or both. This condensation proceeded very readily to yield an aminoalloxazine derivative, which apparently resembles the compound obtained from *m*-phenylenediamine and violuric acid, but the identity could not be proved by mixed melting point since neither of them melt below 350°. However, the compound obtained by Piloty is provisionally considered to be 7-aminoalloxazine.

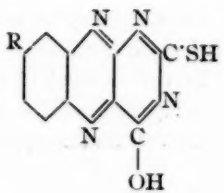
From the fact that *m*-phenylendiamine very readily undergoes condensation with violuric acid to yield an alloxazine, whereas aniline does not, it was surmised that the activation of the *o*-position to the amino group by a substituent in the *m*-position is essential and it was hoped that the *m*-substituted anilines might condense with violuric acid to yield the corresponding alloxazines. But with *m*-toluidine (also *p*-), *m*-aminophenol, *m*-nitroaniline, *m*-aminophenylurea and *m*-aminoacetanilide no condensation product could be isolated, all this yielding blue unstable compounds which are probably the salts of violuric acid with the amines (Zerewitinoff, *Ber.*, 1909, **42**, 4804). *o*- and *p*-phenylenediamines yielded some products which are not alloxazines; β-naphthylamine, wherein the α-position is known to

be very reactive, also did not condense. From these results it appears that the *m*-amino group is absolutely specific for this type of condensation.

Since the sulphur atom present in an organic molecule is known to impart to it specific bactericidal properties and plays a prominent part in the chemotherapy of tuberculosis (it being present in all the gold preparations now current), an attempt was made to introduce the thiol group in the benzene ring of alloxazine. Since this offered some difficulties in the actual working, attention was next directed to the synthesis of compounds of 2-thioalloxazine group (IV, R=H) which can exist in the tautomeric form (V, R=H) so as to form the aurothio (-S. Au) compound with gold salts. 2-Thioalloxazine could not be prepared by condensing *o*-phenylenediamine with 2-thioalloxan, since the latter itself is not yet known (*cf.* Robinson and Tomlison, *J. Chem. Soc.*, 1935, 467). So opportunity was now taken to synthesise a derivative of thioalloxazine by using thiovioluric acid. Thiovioluric acid, similarly as violuric acid, did not condense with aniline, but with *m*-phenylenediamine it furnished an amino-8-thioalloxazine which can be represented as (IV, $R=NH_2$) or its tautomeric form. This compound exhibits properties characteristic of the alloxazines.



(IV)



(V)

EXPERIMENTAL.

Condensation of m-*Phenylenediamine with Violuric Acid.*—The 7-amino-alloxazine was prepared essentially according to the conditions of Piloty (*loc. cit.*). To a boiling solution of *m*-phenylenediamine hydrochloride (0·5 g.) and violuric acid (0·5 g.) in water (15 c.c.) a solution of caustic soda (10%, 2 c.c.) was added whereby the condensation product began to separate as a brick red powder. After boiling for a few minutes, the reaction mixture was cooled, allowed to stand for a few hours, filtered, and the solid washed with a little boiling water, yield 0·8 g. It was obtained from a large volume of water as

a red powder which appears to be crystalline under a high power microscope; not melting at 350°. [Found: N, 30·22; Formula I ($R=NH_2$, $R_1=R_2=H$) $C_{10}H_7O_2N_5$ requires N, 30·57 per cent. Formula (II) $C_{10}H_9O_3N_5$ requires N, 28·34 per cent]. It is fairly soluble in pyridine forming a red solution with a pronounced green fluorescence indicating an alloxazine structure. It is very stable to alkali; after boiling with 15 parts of caustic soda (10%) for 3 hours, a yellow compound crystallising from water in slender yellow needles was obtained which appears to be the sodium salt since on acidifying its aqueous solution the original dyestuff is obtained. Due to the very sparing solubility of the dyestuff, it was difficult to react the compound with benzaldehyde, *iso*cyanates, etc. and isolate the pure reaction products in good yields.

Trimethyl Derivative (III).—Finely powdered aminoalloxazine (1 g.) was allowed to stand with occasional shaking for a day with an ethereal solution (100 c. c.) of diazomethane obtained from nitrosomethylurea (5 g.), the first few hours being in ice and then at the room temperature. The ether was distilled off and the residue so obtained was crystallised from formic acid as microcrystalline red powder not melting at 345°. (Found: N, 25·66. $C_{13}H_{13}O_2N_5$ requires N, 25·82 per cent).

1:2:4-*Triaminobenzene* can very conveniently be prepared as the hydrochloride by the reduction of Bismark brown with tin foil and hydrochloric acid according to the method of Bertds (*Ber.*, 1904, **37**, 2276) or by the reduction of 2 : 4-dinitroaniline (which in its turn was obtained from 2: 5-dinitroacetanilide) with tin and hydrochloric acid (Hinsberg, *Ber.*, 1886, **19**, 1253).

For the preparation of the required 2:4-dinitroacetanilide, the method of Rudnew (*Z. Chem.*, 1871, **7**, 202) yields only 25 g. of the nitrated product from 40 g. of acetanilide. On carrying the nitration at about 30-35°, the nitrated product was obtained only as a gum. After many trials the method described below was found to give the dinitro product as the sole product in very good yield.

Finely powdered acetanilide (20 g.) was gradually added with good stirring to a mixture of fuming nitric acid (*d* 1·51, 500 c.c.) and concentrated sulphuric acid (*d* 1·84 , 200 c.c.) during 45 minutes. By adjusting the rate of addition and cooling the acid mixture with water, the temperature was kept between 30-35°. After the addition of acetanilide was over, the solution was kept stirring for about ½ hour and then slowly poured into ice (about 5 lbs.) when the dinitro compound separated gradually in a yellow crystalline form and collected as a foam. After ½ hour it was filtered,

washed thoroughly with water and dried, yield 27-28 g. It was dissolved in boiling benzene (150 c.c.), rapidly filtered and on allowing the clear green solution to cool, the dinitro compound separated in long needles (24·5 g.). From the mother liquors some more of the product (2 g.) was obtained.

2 : 4-Dinitroacetanilide crystallises from benzene in beautiful yellow needles with a green tinge which becomes very light yellow on drying. It melts at 125-26° (decomp.) (lit. m.p. 120°).

In the course of the hydrolysis to 2 : 4-dinitroaniline it was found that when potassium hydroxide was first added to the acetyl compound and then alcohol, a vigorous reaction took place and after refluxing for about 15 minutes, a product separated which crystallised from water in beautiful golden yellow needles, m.p. about 325° (decomp.). This detonates very violently on heating in a capillary tube in a free flame so that the combustion of this compound was not attempted. It is neither 2 : 4-dinitrophenol nor the quinonoid salt of nitronic acid corresponding to 2 : 4-dinitroaniline described by Green and Rowe (*J. Chem. Soc.*, 1913, **103**, 508). This is being further investigated (*cf.* Cohn and Springer, *Monatsh.*, 1903, **24**, 90).

Condensation of 1 : 2 : 4-*Triaminobenzene and Alloxan.*—On mixing a clear solution of 1 : 2 : 4-triaminobenzene hydrochloride (2 g. in 20 c. c.) containing a little boric acid with a hot solution of alloxan (1·2 g. in 25 c.c.) a turbidity appeared and the condensation product began to separate as a brick-red powder. The whole was warmed on the steam-bath for 5 minutes, cooled, filtered and washed with water, yield 0·9 g. It separated from a large volume of water as a red crystalline powder, not melting at 350°. (Found : N, 30·21. $C_{10}H_7O_2N_5$ requires N, 30·57 per cent). In appearance it resembles the product from *m*-phenylenediamine and violuric acid. Its aqueous solution shows a green fluorescence. It dissolves in alkali to an orange-red solution showing a green fluorescence.

7-*Amino-2-thioalloxazine.*—*m*-Phenylenediamine hydrochloride (1 g.) and thiovioluric acid (1 g.) were dissolved in boiling water (30 c.c.) and to the clear filtered solution aqueous sodium hydroxide (10 %, 4 c.c.) was added and boiled for a few minutes. The well cooled solution was filtered and the brown product obtained was thoroughly washed with hot water, alcohol, ether and dried (0·8 g.). It is a brown crystalline powder not melting up to 340°. (Found : N, 28·22 ; S, 12·76. $C_{10}H_7ON_5S$ requires N, 28·57 ; S, 13·06 per cent). It is very sparingly soluble in all solvents

excepting pyridine in which it shows an intense green fluorescence. It dissolves in alkali (more readily on warming) to an orange solution with a green fluorescence. With concentrated hydrochloric and sulphuric acids, it shows the characteristic red colourations. It is easily desulphurised by yellow oxide of mercury.

The author has great pleasure in thanking Dr. P. C. Guha for his keen interest in the progress of this work. He also thanks the authorities of the Lady Tata Memorial Trust for the award of a scholarship which enabled him to take up this investigation.

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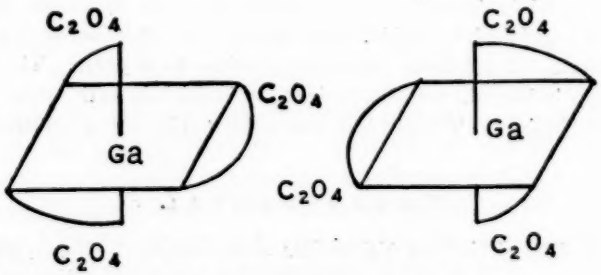
NEW COMPOUNDS OF GALLIUM. PART III. PREPARATION AND RESOLUTION OF COMPLEX OXALATO COMPOUNDS OF GALLIUM INTO OPTICAL ISOMERS.

By Panchanan Neogi and Nihar Kumar Dutt.

The paper describes the preparation and properties of the complex oxalates of gallium with oxalates of sodium, potassium, ammonium and strychnine and also the resolution of the potassium and ammonium salts into their optical isomers by means of the strychnine; preparation of their *d*-components has been described.

Existing literature shows that the complex salts of gallium have not yet been obtained, though double salts, like alums, are well known. As various complex oxalates of trivalent metals like aluminium, chromium and cobalt have been prepared and successfully resolved, it has been our aim to prepare the corresponding compounds of gallium and if possible to resolve them. We have now been able to prepare the sodium, potassium, and ammonium complex oxalates of gallium and have actually resolved the potassium and ammonium salts into their *dextro*-rotatory components by means of strychnine.

The successful resolution of these gallium salts shows that they are complex hexa-co-ordinated compounds with a spatial distribution of the chelate groups round the central gallium atom, and the octahedral structures of the optically active isomers are to be representated as follows :



Wahl (*Ber.*, 1927, **60**, 399) was able to prepare the *d*-variety of only the ammonium salt of the corresponding complex aluminium salt, but we have prepared the *d*-varieties of both the ammonium and potassium salt of the corresponding gallium-oxalato-acid. The first optically active salts of gallium have been obtained by Neogi and Nandi (*J. Indian Chem. Soc.*, 1936, **13**, 399) but this is the first time that a racemic gallium salt has been resolved into optical modification.

The racemic sodium, potassium and ammonium salts were obtained by digesting freshly precipitated gallium hydroxide with the corresponding acid oxalates and their general formula is represented by M_3 [Ga $(C_2O_4)_3$], $3H_2O$, which is analogous to the corresponding aluminium salts. The strychnine salt was prepared by the action of strychnine nitrate on the ammonium salt which is the most soluble of the three when it was obtained as a crystalline precipitate having the formula [Ga $(C_2O_4)_3$] $(H{\cdot}C_{21}H_{22}N_2O_2)_3$, $12H_2O$, also analogous to the corresponding aluminum salt.

The strychnine salt is *laevo*-rotatory with $[M]_D^{33} = -241°$ and consists of *l*-strychnine-*d*-gallium oxalate and *l*-strychnine-*l*-gallium-oxalate. An attempt to separate them by fractional crystallisation from the solution in warm water was unsuccessful as the salt racemises quickly in it They were however, separated by repeated fractional extraction with cold water when owing to differences in solubility of the two isomers in it, the *d*-variety, which was more insoluble, was obtained in the residue, the *l*-component passing in the solution.

It was found that after six fractional extractions of the original substance, the molecular rotation of $-241°$ was changed to $+140°$, which figure remained stationary. The *l*-strychnine-*d*-gallium oxalate was thus obtained.

Having separated the *d*-veriety of the strychnine salt, the *d*-potassium and ammonium salts were obtained by the double decomposition with potassium and ammonium iodides respectively.

The gallium was estimated, after decomposing the complex salt with strong sulphuric acid and precipitating it as hydroxide and then ignited and weighed as oxide. The sodium and potassium were estimated in the filtrates as sulphates after evaporation to dryness. The gallium in the strychnine salt was determined by igniting it until white gallium oxide was obtained. The nitrogen was estimated by Duma's method.

EXPERIMENTAL.

Alkali Salts of Gallium Tri-oxalato Acid, $M_3[Ga(C_2O_4)_3]$, $3H_2O$.

Ammonium Salt.—Equimolecular quantities of ammonium oxalate and oxalic acid were dissolved in water and boiled. The hot solution was then slowly poured over freshly precipitated gallium hydroxide, suspended in water until the major portion of the hydroxide was dissolved leaving behind a small portion of the undissolved hydroxide. This is essentially necessary to ensure the purity of the complex ammonium salt. The hot solution was filtered, concentrated on the water-bath and then allowed to crystallise

in a desiccator when the salt was obtained in beautiful crystals. The salt is very easily soluble in water and insoluble in alcohol. The substance is stable in the solid state as well as in solution. {Found : N, 9·5, 9·48 ; Ga, 15·2, 15·1. $(NH_4)_3[Ga(C_2O_4)_3]$, $3H_2O$ requires N, 9·5; Ga, 15·8 per cent}.

Potassium salt was obtained very similarly to the ammonium salt substituting ammonium oxalate with potassium oxalate. It resembles the ammonium salt in solubility and other properties though the ammonium salt is more soluble in water. {Found : Ga, 13·9, 13·8; K, 23·2, 23·1. $K_3[Ga(C_2O_4)_3]$, $3H_2O$ requires Ga, 13·8 ; K, 23·2 per cent}.

Sodium salt was obtained as above using sodium oxalate in the place of ammomium oxalate. In solubility and other properties it resembles the potassium salt. {Found : Ga, 15·2, 15·2 ; Na, 15·01, 15·2. $Na_3[Ga(C_2O_4)_3]$ $3H_2O$ requires Ga, 15·2 ; Na, 15·0 per cent}.

l-*Strychnine Gallium Trioxalate*, l-$[Ga(C_2O_4)_3]$ $(HC_{21}H_{22}O_2N_2)_3$, $12H_2O$.—The strychnine salt was obtained by precipitating a cold saturated solution of strychnine nitrate with a cold saturated solution of the ammonium salt of gallium trioxalato-acid. The precipitate was filtered, washed and dried. The salt is slightly soluble in cold water, easily soluble in warm water and in alcohol. In 1% solution of the salt in aqueous alcohol (1 : 1) in a 2 dm. tube, $[\alpha]_D^{33} = -15·5°$ $[M]_D^{33} = -242°$. {(Found : N, 5·5, 5·47; Ga, 4·0, 4·6. $[Ga(C_2O_4)_3]$ $(H·C_{21}H_{22}O_2N_2)_3$, $12H_2O$ requires 4·5 ; 5·4 per cent)}.

Preparation of l-*Strychnine*-d-*gallium Oxalate from* l-*Strychnine-gallium Oxalate.*

The process of fractional extraction was employed as was done by Wahl (*loc. cit.*) in the case of the corresponding aluminium salt. For extraction 20 g. of the powdered salt were shaken with cold distilled water, filtered and the residue dried between filter papers and finally on a porous tile.

The results of an extraction are given below and 1% solution in (1 : 1) alcohol was examined in the polarimeter in a 2 dm. tube.

		$[\alpha]_D^{33}$.	$[M]_D^{33}$.
Original substance		−15·5°	−241·1°
1. Residue after extraction with	100 c c. of H_2O	− 15°	−233·2°
2. „	300	− 9·5°	−147·7°
3. „	106	− 8·5°	−132·2°

			$[\alpha]_D^{28}$.	$[M]_D^{28}$.
4.	Residue after extraction with	200 c.c. H_2O	− 6°	−93·3°
5.	„ „	100	−4·5°	−71·6°
6.	„ „	100	+ 9°	+ 140°
7.	„ „	100	+ 9°	+ 140°

The specific rotation of the *l*-strychnine-*d*-gallium oxalate was therefore +29° and $[M]_D^{28} = +140°$.

Preparation of d-*Potassium and* d-*Ammonium Gallium Oxalate.*

Dextro-varieties of the potassium and ammonium salts were obtained by the double decomposition of the strychnine salt with the solutions of the corresponding iodides. Half the quantity of the *d*-strychnine salt was treated with KI and the other half with NH_4I. A weighed quantity of the strychnine salt was taken in a mortar and the theoretical quantity of KI was dissolved in water and added to it and triturated. Strychnine iodide was precipitated and the solution tested for any excesss of KI. The solution was then filtered and to the filtrate was added a large excess of alcohol and cooled. The salt was precipitated, filtered, and immediately dried under an electric fan.

Similarly, the ammonium salt was obtained by using ammonium iodide.

	Salt.	Gallium Found.	Gallium Req.	$[\alpha]_D^{28}$.	$[M]_D^{28}$.
I	*d*-K_3 [Ga $(C_2O_4)_3$], $3H_2O$	13·6	13·8	+ 16·5°	+ 83·3°
II	*d*-$(NH_4)_3$ [$Ga(C_2O_4)_3$], $3H_2O$	15·2	15·8	+ 15·5°	+ 68·5°

Racemisation of the Active Potassium and Ammonium Salts.

These active salts of potassium and ammonium gradually became racemic in a short time. The following table will show the rate of racemisation.

d-K.	$[\alpha]_D^{28}$.	$[M]_D^{28}$.
Original reading	+ 16·5°	+ 83·3°
Reading after 2 hours	+ 14°	+ 67·5°
27 „	+ 4°	+ 20·1°
d-NH_4.		
Original reading	+ 15·5	+ 68·5°
Reading after 3 hours	+ 12·5°	+ 55·3°
27 „	+ 3°	+ 13·3°

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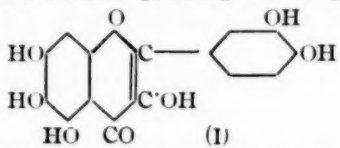
QUERCETAGETIN FROM THE FLOWERS OF *TAGETES ERECTA*.

By Harbhajan Singh Mahal.

Quercetagetin has been isolated from French marigold (Ganda) of Indian variety.

Quercetagetin (I), the yellow pigment of the flowers of *Tagetes patula* (the African marigold), was isolated by Latour and de la Source (*Bull. Soc. Chim.*, 1877, *ii*, **28**, 337; Perkin and Everest "The Natural Organic Colouring Matters", p. 230), according to whom it also occurs in other varieties of the plant whose names, however, are not mentioned in the paper. Perkin (*Proc. Chem. Soc.*, 1902, **18**, 75) obtained the colouring matter in the pure state and showed that quercetagetin was 3:5:6:7:3′:4′-(or 3:5:7:8:3′:4′)-



(I)

hexahydroxyflavone, the correctness of the first suggestion (and the identity of gossypetin with the second) being more recently demonstrated by synthesis (Baker, Nodzu and Robinson, *J. Chem. Soc.*, 1929, 74).

The flowers of *Tagates erecta* (the French marigold ; *Hindi*, Ganda), a plant commonly cultivated in gardens throughout India, have now been examined and both the yellow and orange flowers shown to contain the same pigment as *Tagetes patula*. The substance is readily obtainable by the extraction method described by Perkin (*loc. cit.*), but a preliminary treatment with chloroform gave a better yield of more easily crystallisable material. The juice of the flowers of *Tagetes erecta* is reputed to have pharmacological properties as a purifier of the blood and as a remedy in piles (Nadkarni, "Indian Materia Medica", p. 834).

Experimental.

Immediately after collection the petals (1800 g.) of the orange flowers were carefully removed, dried in the air and extracted with chloroform in a Soxhlet for an hour. The colouring matter was then extracted with alcohol, the extraction being continued until the extract was colourless. The alcoholic solution was then reduced in bulk to 200 c.c. under diminished pressure and filtered hot to remove the dark brown resinous matter which had separated. After cooling the clear filtrate was mixed with ether and water, the ether layer separated and the ether removed. The bright yellow residue (13 g.) was acetylated by means of acetic anhydride and pyridine.

The product isolated on admixture with water was dark brown in colour, but when left in contact with glacial acetic acid overnight, the substance was obtained as a colourless, crystalline powder. Crystallisation from alcohol-acetic acid, followed by recrystallisation from methanol, gave colourless silky needles (7·5 g.), m.p. 210°. (Found : C, 56·7 ; H, 3·9. Calc. for $C_{27}H_{22}O_{14}$: C, 56·8 ; H, 3·9 per cent). Hydrolysis of the acetate (7 g.) was effected by dissolution in boiling glacial acetic acid (50 c.c.) and graduai addition of concentrated hydrochloric acid (50 c.c.). Boiling water (100 c.c.) was then added and, on cooling, quercetagetin separated in long, silky, pale brownish yellow needles (3·5 g.). m. p. 317° (decomp.). The substance crystallises from alcohol with two molecules of water [Found by heating at 160° for 3 hours : H_2O, 9·9. $C_{15}H_{10}O_8$, $2H_2O$ requires H_2O, 10·1 per cent. Found (in the dried material) : C, 56·5; H, 3·4. Calc. for $C_{15}H_{10}O_8$: C, 56·5 ; H, 3·2 per cent]. The pure substance is readily soluble in boiling alcohol and sparingly soluble in boiling water in which the impure pigment is fairly soluble. The alcoholic solution of the flavone exhibits an olive green colouration with ferric chloride ; with lead acetate an orange-red precipitate is formed, which gradually turns yellow and finally green. The brownish yellow solution in concentrated sulphuric acid develops a bluish green fluorescence on standing.

Quercetagetin Hxamethylether.—To a mixture of the hexacetate (6·5 g.) and acetone (50 c.c.) in a three-necked flask equipped with a mercury-seal stirrer, an inlet and outlet for hydrogen and two dropping funnels, dimethyl sulphate (375 g.) and 50% aqueous caustic soda (154 g.) were added simultaneously drop by drop, the reaction mixture being tested for alkalinity from time to time. During the addition of the first 100 c.c. the mixture was yellow in colour and a dark green oil separated at the bottom. Further addition of methyl sulphate and alkali initiated a vigorous reaction, which rapidly became violent and was controlled by cooling in running water. After the complete addition of the reagents (3 hours), the whole reaction mixture became clear, pale orange ; the stirring was continued for 30 minutes and on pouring into water (1 litre) the bright yellow product was collected and twice crystallised from acetone in long, pale yellow needles, m. p. 142°, yield 1·5 g. (Found : C, 62·3 ; H, 5·3. Calc. for $C_{21}H_{22}O_8$: C, 62·7 ; H, 5·5 per cent). When the molten substance solidified and the m.p. was again observed it was found to be 155° (*cf.* Perkin *loc. cit.* ; Baker, Nodzu and Robinson, *loc. cit*).

I am indebted to Dr. K. Venkataraman, D.Sc., F.I.C., for his interest in this work.

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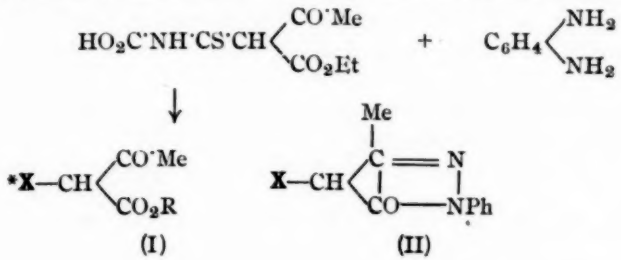
QUINOLINE DERIVATIVES. PART IV.

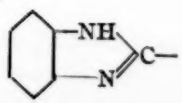
By Tejendra Nath Ghosh.

In view of the fact that glyoxalinoquinolines of Narang and Rây have the requisite antiseptic properties against paramaecia, benziminazolylquinoline derivatives, which are expected to possess antimalarial properties, have been synthesised

Glyoxalinoquinoline, synthesised by Narang and Rây (*J. Chem. Soc.*, 1931, 976), has the requisite antiseptic properties as tested against paramaecia in a dilution of 1 : 1000, but the compounds of this series have very feeble basicity and do not give stable salts (*cf.* Rây, Presidential Address, *Proc. Indian Sci. Congress*, 1937, p. 103). In view of this important observation, it seemed of considerable interest to synthesise some benziminazolylquinoline derivatives, which are expected to possess antimalarial properties. The presence of the benziminazole residue is likely to impart pronounced basicity to these products.

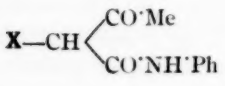
In a previous communication (Ghosh, *J. Indian Chem. Soc*, 1936, **13**, 89) it has been shown that the condensation of thioacetylcarbamic acid derivatives with diamines constitutes a general method for the synthesis of iminazole derivatives. Following the same method, the benziminazole derivative (I, R=Et) has now been synthesised by reacting acetylcarbethoxythioacetocarbamic acid with *o*-pheynlenediamine. Its constitution has been established by its conversion to 2-methylbenziminazole (Hübner, *Annalen*, 1881, **209**, 353) by alkali hydrolysis. With concentrated hydrochloric acid it yields the acid (I, R=H). Both these compounds (I, R=H



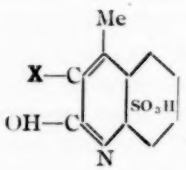
* In the formulæ in this paper **X** stands for 

or Et) react with phenylhdrazine to yield the benziminazolylpyrazolone derivative (II). Because of the presence of both iminazole and pyrazolone rings, the compound (II) is expected to exihibit important therapeutic properties.

The compound (I, R=Et) readily condenses with aniline at 180° to yield the anilide (III). When treated with concentrated sulphuric acid at 100° the compound (III) furnishes the benziminazolylquinoline derivative (IV) (*cf.* Knorr, *Ber.*, 1892, **25**, 772). It is, however, to be observed that, during the treatment with concentrated sulphuric acid, the resulting product (IV) gets sulphonated.

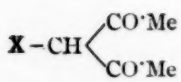


(III)

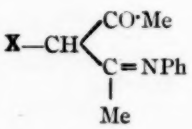


(IV)

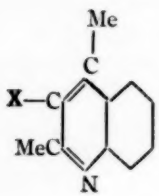
Acetylacetonanilide, obtained by condensing acetylacetone with aniline, yields 2:4-dimethylquinoline, when treated with sulphuric acid (Combes, *Compt. rend.*, 1887, **106**, 142 ; *Bull. Soc. chim.*, 1888, **49**, 90). Taking advantage of this reaction, the benziminazole derivative (V), previously synthesised (Ghosh, *loc. cit.*), has now been condensed with aniline at 145-150° to yield (VI). When treated with concentrated sulphuric acid at 100°, the compound (VI) yields the benziminazolylquinoline derivatfve (VII).



(V)

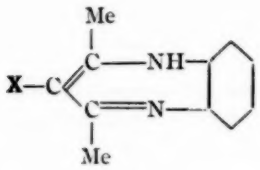


(VI)



(VII)

When, however, condensed with *o*-phenylenediamine at 150°, the compound (V) yields (VIII) with elimination of two molecules of water (*cf.* Thiele and Steimmig, *Ber.*, 1907, **40**, 955).



(VIII)

As regards the basicity of the two benziminazolyl quinoline derivatives, (IV) and (VII), the former forms a stable hydrochloride, which is very soluble in cold water and is, thus, suitable for pharmacological examination. The latter also yields a hydrochloride which is very soluble in cold water, but the aqueous solution slowly precipitates the base.

EXPERIMENTAL.

2-Carbethoxyacetylmethylbenziminazole (I, R = Et).—To an alcoholic solution of acetylcarbethoxythioacetocarbamic acid (23·3 g.; prepared according to the method of Ghosh and Guha, *J. Indian Inst. Sci.*, 1933, **16A**, 107), *o*-phenylenediamine (10·8 g.) was slowly added, when the reaction at once began with evolution of sulphuretted hydrogen and rise in temperature. The solution was heated under reflux for about 2 hours, cooled and diluted with water, when a semi-solid mass was obtained. It was purified by dissolving in dilute hydrochloric acid and precipitating with excess of sodium bicarbonate solution. The solid, thus obtained, was crystallised from hot water in colourless needles, m. p. 128-29°, yield 14 g. (Found : N, 11·12. $C_{13}H_{14}O_3N_2$ requires N, 11·38 per cent). It is insoluble in aqueous sodium bicarbonate solution, but readily soluble in cold dilute hydrochloric acid and also in dilute alkali.

A solution of the above compound in excess of alcoholic potash (15%) was heated under reflux for about 3 hours and the solution was evaporated nearly to dryness on a water-bath. The aqueous solution of the mass was acidified with acetic acid and filtered. The acid solution was treated with excess of sodium bicarbonate ; the solution, on standing, gave a precipitate

tate which crystallised from water in colourless needles, m. p. 170° (mixed m. p. with 2-methylbenziminazole, Hübner, *loc. cit.*). (Found : N, 21·37. $C_8H_8N_2$ requires N, 21·21 per cent).

2-Carboxyacetylmethylbenziminazole (I, R=H).—A solution of the above compound in concentrated hydrochloric acid was boiled for 30 minutes, diluted with water and the solution evaporated to a small bulk. On cooling, a crystalline solid (hydrochloride) was obtained, which crystallised from water, acidulated with few drops of hydrochloric acid, in colourless rectangular plates, m. p. 290-91°. An aqueous solution of this hydrochloride was treated with excess of sodium acetate, when a solid was precipitated, which crystallised from water in colourless rectangular plates, m. p. 172°. (Found : N, 12·61. $C_{11}H_{10}O_3N_2$ requires N, 12·84 per cent). It is readily soluble in aqueous sodium bicarbonate solution.

1-Phenyl-3-methyl-4-(2′)-benziminazolylpyarzolone (II).—A glacial acetic acid solution of the compound (I, R=Et; 2·5 g.) and phenylhydrazine (1·1 g.) was heated under reflux for about 2 hours. The solution was diluted with water and evaporated to a small bulk. When treated with excess of sodium bicarbonate solution, a solid was obtained which was purified by dissolving in cold dilute hydrochloric acid and precipitating with sodium bicarbonate solution. It crystallised from hot water in colourless rectangular plates, m p. 172-73°, yield 1 g. (Found : N, 19·28. $C_{17}H_{14}ON_4$ requires N, 19·31 per cent). It is readily soluble in cold dilute alkali. A solution of the compound in cold dilute hydrochloric acid was evaporated to a small bulk, when, on cooling, the hydrochloride was obtained, which crystallised from a small quantity of water in almost colourless rectangular plates, m. p. 282-84°. It is readily soluble in cold water.

The same compound (m. p. 172-73°) was obtained, when the compound (I, R=H) was allowed to react with phenylhydrazine under similar conditions Identity was confirmed by mixed m.p. and other properties.

Condensation of the Compound (I, R=Et) *with Aniline : Formation of the Anilide* (III).—A mixture of the compound (I, R=Et ; 7·4 g.) and aniline (2·8 g.) was heated at 170° for about 1 hour. The solid obtained was cooled, triturated with cold alcohol and filtered. It crystallised from alcohol (charcoal) in colourless shining plates. m p. 255° (decomp., producing a bright red colouration), yield 5·5 g. (Found : N, 14·21. $C_{17}H_{15}O_2N_3$ requires N, 14·33 per cent). It is insoluble in cold dilute alkali.

2 - Hydroxy - 3 - (2′) - benziminazolyl - 4 - methylquinoline-sulphonic Acid (IV).—A solution of the above compound (III ; 5 g.) in concen-

trated sulphuric acid (33 c. c.) was heated on an oil-bath at 100° for 1½ hours. When cooled, the reaction mixture was poured into ice-cold water, when a brown solid (4 g.) was obtained. It was filtered and washed several times with alcohol. It was purified by dissolving in aqueous bicarbonate solution and precipitating with dilute hydrochloric acid. It is insoluble in hot alcohol or glacial acetic acid. It is sparingly soluble in boiling water, from which it was crystallised (charcoal) in colourless plates, m. p. 293-95° (decomp.). [Found : N, 11·72; S, 8·53; M. W. (by titration), 358. $C_{17}H_{13}O_4N_3S$ requires N, 11·83; S, 9·01 per cent. M. W., 355].

The clear solution, obtained by heating the above compound with concentrated hydrochloric acid, was diluted with water and evaporated to a small bulk, when the hydrochloride was obtained. It crystallised from water in colourless needles, which turned grey above 240° and did not melt even at 310°. [Found (in a sample dried at 120°): N, 10·48. $C_{17}H_{13}O_4N_3S$, HCl requires N, 10·74 per cent). It is readily soluble in cold water and remains stable in solution. The aqueous solution, when treated with excess of sodium acetate, precipitates the above compound (IV mixed m. p. 293-95°).

δ-Phenylimido-γ-(2′)-benziminazolyl-β-ketopentane (VI).—A mixture of aniline (9·3 g.) and 2-diacetylmethylbenziminazole (21·6 g., prepared according to the method of Ghosh, *loc. cit.*) was heated on an oil-bath at 145°-50° for 2 hours, when there was elimination of water vapour. The cold molten mass was triturated with benzene, when a solid was obtained which was filtered and washed with benzene. It is practically insoluble in alcohol and crystallises from glacial acetic acid in colourless rectangular plates, m. p. 315-16° (decomp., producing a red colouration), yield 8 g. (Found: N, 14·21. $C_{18}H_{17}ON_3$ requires N, 14·43 per cent). It is insoluble in dilute hydrochloric acid but readily soluble in cold concentrated acid.

2:4-*Dimethyl*-3-(2′)-*benziminazolylquinoline* (VII).—A solution of the above compound (VI, 6 g.) in concentrated sulphuric acid (35 c.c.) was heated on an oil-bath at 100° for 30 minutes, cooled and poured into ice-cold water. The clear acid solution was treated with excess of sodium bicarbonate, when a solid was precipitated. It is readily soluble in acetic acid and crystallises from a large quantity of alcohol in colourless needles, m.p. 328-30° (producing a red colouration), yield 3 g. (Found: N, 15·11. $C_{18}H_{15}N_3$ requires N, 15·38 per cent). The *picrate* was obtained as yellow needles, m.p. 250° (decomp., producing a deep red colouration).

The above compound (VII) was dissolved in cold dilute hydrochloric acid; the acid solution on evaporation to a small bulk yielded the hydrochloride as colourless rectangular plates, m. p. above 300°. It is readily soluble in cold water; the aqueous solution on standing precipitates the base (mixed m. p. 328-30°).

2:4-*Dimethyl*-3-(2')-*benziminazolyl*-6:7-*benzo*-1:5-*heptadiazine* (VIII) — An intimate mixture of *o*-phenylenediamine (3·6 g.) and 2-diacetylmethylbenziminazole (7·2 g.) was heated at 150° for 2 hours. The temperature was raised to 180° for about 10 minutes and the mass allowed to cool. It was triturated with benzene, when a solid was obtained which crystallised from alcohol in yellowish rectangular plates, m. p. above 310°, yield 3 g. (Found: N, 19·42. $C_{18}H_{16}N_4$ requires N, 19·44 per cent). It does not contain any diazotisable amino group. It is readily soluble in cold dilute hydrochloric acid; the acid solution, on evaporotion to a small bulk, yielded the hydrochloride in the form of colourless rectangular plates, m. p. above 300°. This hydrochloride is readily soluble in cold water.

My thanks are due to Prof. P. C. Guha for his kind interest in this investigation. My thanks are also due to the Lady Tata Memorial Trust for the award of a scholarship.

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CHEMICAL EXAMINATION OF THE SEEDS OF *SOLANUM XANTHOCARPUM*, (SCHARD AND WENDLE). PART II. THE CONSTITUENTS.

By Mahadeo Prasad Gupta and Sikhibhushan Dutt.

The benzene extract of the seeds of *Solanum xanthocarpum* yields (*i*) semi-drying oil, (*ii*) a lactone, $C_{28}H_{42}O_7$, m. p. 78° and (*iii*) a sterol, carpesterol, $C_{36}H_{54}O_4$, m. p. 216° $[\alpha]_D^{30} = -80°$ in $CHCl_3$. The alcoholic percolate of the defatted seeds gives in addition to the lactone, a gluco-alkaloid, $C_{44}H_{74}O_4N_2$, m. p. 272°, $[\alpha]_D^{20} = +83\ 53°$ which on acid hydrolysis gives the aglucone, solanacarpigenin, $C_{32}H_{54}O_2N_2$, m. p. 96° $[\alpha]_D^{20} = +88·7$ and glucose, rhamnose and potassium chloride.

Solanum xanthocarpum as a whole plant was examined by Pendse and Dutt (*Indian J. Med. Res.*, 1932, **20**, 663) but they could not isolate any alkaloid although they reported the presence of it in the plant in traces. They attributed the physiological activity of the whole plant to potassium nitrate which is present in it to the extent of 1·6%.

Saiyed and Kanga (*Proc. Indian Sci. Congress*, 1934, p. 44) reported the isolation of a gluco-alkoloid (m. p. 245°) from the seeds of the plant by petrol extraction. In addition to this they also extracted two more basic substances melting at 265° and 196°. In a later communication (*Proc. Indian Acad. Sci.*, 1936, **4A**, 255) the same authors have given the results of the examination in detail, where they claim to have isolated a gluco-alkaloid *solanacarpine* ($C_{42}H_{77}O_{19}N$), m.p. 288-89° (decomp.) in white flat needles, and an alkaloid called *solanacarpidine* ($C_{26}H_{43}O_3N$), m. p. 197-98°, in the form of shining plates, from the alcoholic extract of the fruit. They have also isolated a sterol named by them *carpesterol*, m. p. 248°, which they had previously reported as a gluco-alkaloid, and a fixed oil from the petrol extract of the fruit.

Amongst the products of hydrolysis of the above gluco-alkaloid ($C_{44}H_{77}O_{19}N$) they have identified the alkaloid $C_{26}H_{43}O_3N$, and glucose, rhamnose and galactose, the presence of the latter being doubtful. They also state that the sugar-free alkaloid ($C_{26}H_{43}O_3N$) is present in the fruit as such, although they have not recorded any method for the separation of the two.

The present investigation was already in progress when the above paper by Saiyed and Kanga was published. On account of the great

medicinal importance of the plant in the indigenous system of medicines, and also because of the fact that one of the present authors had already tackled the whole plant before, it was highly desirable that the investigation on the seeds of the plant should be more thoroughly carried out.

In a previous communication Gupta and Dutt (*J. Indian Chem. Soc.*, 1936, **13**, 613) in course of their investigation on the chemical constituents of the seeds of *Solanum xanthocarpum*, isolated from the benzene extract, a fixed oil which on standing deposited some crystalline substance. In the present paper, the systematic chemical analysis of this substance as well as the isolation and examination of the other constituents of the seeds are given.

The above mentioned crystalline substance on further treatment was separated into a sterol, *carpesterol* (m.p. 248°) isolated by the previous workers, with a molecular formula $C_{36}H_{54}O_4$, and another microcrystalline substance ($C_{28}H_{42}O_7$, m.p. 78°) which exhibited all the characteristic properties of the $\Delta^{\alpha\beta}$-unsaturated lactones.

The present authors have also isolated from the alcoholic extract some more of the lactone stated above, and also a gluco-alkaloid, $C_{44}H_{74}O_{11}N_2$, m.p. 272° crystallising in snow-white needles, and not the sugar-free alkaloid as the previous authors claim. Besides that, the present alkaloid is found to contain two atoms of nitrogen in the molecule instead of one atom as found by the previous workers. An alkaloid, $C_{32}H_{54}O_2N_2$, m.p. 196°, together with glucose and rhamnose have been identified as the products of hydrolysis of the gluco-alkaloid by mineral acids. There is also obtained some inorganic salt which has been identified as potassium chloride.

Further examination of the seeds of the plant for the sugar-free alkaloid has revealed the interesting fact that it is not present in the seeds as such, and the fact that the previous workers isolated it from the seeds may be due to the hydrolysing effect of hydrogen sulphide which they used in course of the isolation.

Between the two methods, namely the lead salt method, and the chromate salt method that have been worked up for the isolation of the gluco-alkaloid, the latter is most satisfactory and convenient, and the yield of the gluco-alkaloid is also quite good. Unlike the former, in the latter process no resinification or hydrolysis of the gluco-alkaloid takes place and the guco-alkaloid is obtained in a pure condition.

Contrary to the statement of Saiyed and Kanga (*loc. cit.*) we find that the gluco-alkaloid can not be crystallised from pure ethyl alcohol, as it does not separate from dilute solution, and from hot concentrated solution it

separates as a translucent jelly on cooling. It has been crystallised by the present authors from a mixture of alcohol and ether.

A sample of the alkaloid has been sent to the Tropical School of Medicine, Calcutta for physiological investigation. Further work on the constitution of the alkaloid is in progress and will be dealt with in a separate communication.

EXPERIMENTAL.

Preliminary Experiments.—After a series of preliminary experiments, 8·2 kg. of the powdered and oil-free seeds were percolated 6 times with hot 98% ethyl alcohol in two lots. The combined percolates were concentrated to half their original volume at the ordinary pressure and allowed to stand overnight, when a quantity of colourless crystalline matter was deposited which was filtered off and identified as potassium chloride. The filtrate was further concentrated under diminished pressure below 40° and the resulting brownish yellow syrup completely dried in a vacuum desiccator over calcium chloride. The viscid residue was finally freed from traces of oil by thorough agitation with petroleum ether.

Isolation of the Gluco-alkaloid Solanacarpine.—The above dried residue was extracted with 5% acetic acid a number of times until the last extract gave no appreciable precipitate with ammonia; the voluminous precipitate was filtered off and dried. This was then further extracted with 2% dilute hydrochloric acid and the filtered extract basified with ammonia when a dirty grey precipitate was obtained which was filtered off, washed and dried. The crude alkaloid thus obtained could not be crystallised from any solvent, and the only insoluble salts that could be obtained from it were the lead salt and the chromate salt. As the lead compound necessitated the use of hydrogen sulphide for its decomposition, which invariably complicated matters by extensive hydrolysis of the gluco-alkaloid, the chromate salt was found to be the most suitable for the purification of the gluco-alkaloid. A solution of potassium chromate was added to dilute acetic acid solution of the crude alkaloid, and the yellow amorphous voluminous precipitate of the chromate was filtered off, washed with water and then crystallised from alcohol. The crystalline chromate was then suspended in water and decomposed with excess of aqueous ammonia, when a dirty white precipitate of the impure gluco-alkaloid was obtained. This was dissolved in alcohol and boiled with animal charcoal and the alcoholic filtrate concentrated when an almost colourless translucent jelly was ob-

tained. On allowing to stand much of the mother liquor separated from the stiff translucent material and the latter was filtered off and dried. The dried product was then dissolved in alcohol and ether gradually added, when most of the impurities separated in the form of a brownish viscous mass. The product was filtered at this stage and more ether added to the filtrate until a slight turbidity appeared. On allowing to stand, the gluco-alkaloid separated in the form of glistening colourless curved needles, which under the microscope have a characteristic feathery appearance. On heating the substance begins to darken in colour at 249°, becomes completely brown at 262° and melts at 272°. It is optically active, $[\alpha]_D^{30} = +83{\cdot}5°$. The yield of the gluco-alkaloid was 1·41% on the weight of the dried seeds. (Found : C, 65·23, 65·05, 64·96 ; H, 8·95, 9·00, 9·28 ; N, 3·50, 3·14. $C_{44}H_{74}O_{11}N_2$ requires C, 65·50 ; H, 9·18 ; N, 3·59 per cent).

The gluco-alkaloid is soluble in water and dilute acids. It is readily soluble in ethyl and methyl alcohol, chloroform and acetone, sparingly soluble in ethyl acetate, and insoluble in benzene, ether and petroleum ether. It dissolves in concentrated sulphuric acid with a bright yellow colour which gradually changes to orange and red, and on keeping becomes dark brown. It dissolves with a yellow colour in cold concentrated nitric acid which remains unchanged on standing.

Hydrolysis of the Gluco-alkaloid and Isolation of the Aglucone: Solanacarpigenin.—The chromate salt of the gluco-alkaloid (50 g.) was heated with excess of 5% hydrochloric acid under reflux for 3 hours on a boiling water-bath. The separated dirty solid was filtered, washed, dried and decomposed with ammonia in alcoholic solution. After filtration, the liquid was concentrated, when the aglucone separated in the form of a brown crystalline mass. This was filtered off, the residue extracted with ether, and the ethereal extract evaporated. The colourless crystalline mass was then recrystallised from alcohol as glistening snow-white star-shaped prisms, m.p. 196°. The yield of the alkaloid was 0·35% on the weight of the dry seeds. (Found : C, 76·49, 76·96 ; H, 10·63, 10·94 ; N, 5·50, 5·78. $C_{32}H_{54}O_2N_2$ requires C, 77·10 ; H, 10·84 ; N, 5·62 per cent).

This aglucone has solubilities quite similar to the gluco-alkaloid. It is optically active, $[\alpha]_D^{30} = +88{\cdot}79°$. With concentrated sulphuric acid it produces a bright yellow colouration, which gradually turns orange, then red and finally dark brown. On gradually adding water the colouration changes to green, then blue, then yellow and ultimately disappears. With concentrated nitric acid it produces a colouration similar to the gluco-alkaloid.

The filtrate left after the acid hydrolysis of the chromate salt of the gluco-alkaloid and removal of the aglucone, contained reducing sugars which were converted into their mixed osazones and separated by repeated crystallisations from acetone in which they had different solubilities. In this way the presence of glucose and rhamnose was confirmed.

Isolation of the Lactone : Solanocarpone.

(*a*) *From the Oil-free Seed.*—The residue left after treatment with 5% acetic acid in course of isolation of solanacarpine was digested with caustic soda in the cold a number of times, and the combined extracts left overnight whereby some resinous impurities settled down which were filtered off. The filtrate on acidification with dilute hydrochloric acid deposited the lactone as a brown precipitate which on crystallisation from methyl alcohol was obtained in cream coloured microscopic needles, m.p. 78°.

(*b*) *From the Oil.*—The oil on allowing to stand for about a month deposited some crystalline matter which was filtered off, washed with petroleum ether, dried and recrystallised from benzene until free from stickiness. The substance was then repeatedly extracted with warm ethyl alcohol until nothing further was extracted. The combined extract on cooling deposited the lactone as a brown amorphous powder which on crystallisation from boiling absolute alcohol was obtained as a cream coloured mass of microscopic needles, m.p 78°, yield 2·1 g. or 0·12% on the weight of the dry seeds. [Found : C, 68·40, 68·86 ; H, 9·29, 9·00 ; M.W. (ebullioscopic in ethyl alcohol), 460,553. $C_{28}H_{42}O_7$ requires C, 68·57 ; H, 8·57 per cent. M.W. 490].

The substance shows all the characteristic properties of $\Delta^{\alpha\beta}$-unsat :rated lactones. It dissolves easily in benzene, acetone, ethyl acetate, chloroform and phenol; it is slightly soluble in methyl and ethyl alcohol and is insoluble in ether, petroleum ether and water. It dissolves in alcoholic caustic soda or potash with an intense yellow colouration, from which it is precipitated unchanged on acidification. It quickly decolourises a solution of bromine in chloroform and also an alkaline and acidic solution of potassium permanganate. It gives a positive Salkowski's reaction, *i.e.*, a solution of the lactone in chloroform gives with concentrated sulphuric acid a red and finally a green colouration. A solution of the lactone in chloroform on treatment with acetic anhydride followed by concentrated sulphuric acid gives a fine violet colouration. With concentrated sulphuric acid it produces an intense yellow colour which finally changes to deep red. With

concentrated nitric acid it develops a yellow colour on heating. It gives a white precipitate with lead acetate, but no precipitate or colouration with silver nitrate or ferric chloride. With Tollen's reagent a light yellow colouration is formed which slowly changes to brown and then a gradual reduction takes place. With an alkaline solution of sodium nitroprusside it gives no colouration.

Isolation of a Carpesterol from the Oil.—The crystalline deposit from the oil after separation of the above lactone, was dissolved in boiling ethyl alcohol and on cooling the sterol separated in the form of shining white plates, m.p. 248°, $[\alpha]_D^{30} = -80°$. (Found : C, 78·46, 78·1 ; H, 10·1, 9·4. $C_{36}H_{54}O_4$ requires C, 78·54 ; H, 9·81 per cent).

The substance is fairly soluble in boiling ethyl and methyl alcohol, sparingly soluble in hot benzene, chloroform and acetone and very sparingly soluble in ether and petroleum ether. It gives all the characteristic colour reactions of a sterol.

The *acetyl* derivative was obtained by the usual methods and it crystallised from alcohol in colourless silky needles, m.p. 194°. (Found : C, 77·20, 76·98 ; H, 9·91, 10·12. $C_{38}H_{56}O_5$ requires C, 77·03 ; H, 9·5 per cent).

The *benzoyl* derivative was obtained by benzoylation with benzoyl chloride and pyridine in the usual manner and crystallised from ethyl alcohol in colourless silky needles, m.p. 216°. (Found : C, 78·52 ; H, 9·12. $C_{43}H_{58}O_5$ requires C, 78·89 ; H, 8·86 per cent).

One of the authors (M.P.G.) wishes to express his indebtedness to the Kanta Prasad Research Trust of the Allahabad University for a scholarship which enabled him to take part in this investigation.

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STUDIES ON THE ENZYMES OF THE SEEDS OF *BUTEA FRONDOSA*. PART I. PROTEOLYTIC ENZYME.

By N. R. Chatterjee, S. Ghosh and R. N. Chopra.

The fresh seeds of *Butea frondosa* possess anthelmintic action, specially against ascaris. The seeds were found to contain proteolytic enzymes which hydrolyse peptones and casein, liquefy gelatin but have no action on egg-albumin or fibrin. They act best in a slightly alkaline medium and the optimum temperature is near about 40°. Hydrocyanic acid is found to have a definite activating influence. They behave like 'yeast trypsin' and appear to be a mixture of plant proteinase and polypeptidase.

The various parts of the common Indian plant *Butea frondosa*, like the bark, gum, leaves, flowers and seeds, have been used in the Indian indigenous medicine in various ailments from the very earliest times. The seeds are said to be aperient and anthelmintic, used with success in tape-worms and round-worms. Externally, the seeds are said to be useful in Dhobie's itch, ringworm, ulcers and fistula (*cf.* B. C. Gupta, "Ayurvedic Materia Medica", 1908, **II**, p. 54). As regards the therapeutic action, it is stated that the old worm-eaten seeds show little activity but freshly powdered seeds give fairly good results against ascaris (*cf.* Chopra, "Indigenous Drugs of India", 1933, p. 306).

A fair amount of chemical work has been carried out on all the constituents of the flowers, and some work is also reported on the gum. The seeds are reported to contain fatty oil, glucose, saccharose, starch, gum, phlobaphenes, proteins, etc. (Waber, *Pharm. Z. Ruszland*, 1896, 429; Heckel, "Graines grasses nouvelles des colonies francaises," Paris, 1902, 98). The most recent work on the seeds shows the presence of 18 % of fatty oil composed of glycerides of oleic, linolic, palmitic, lignoceric and other acids and the non-saponifiable matter has been found to contain sitosterol (Tummin Katti and Manjunath, *J. Indian Chem. Soc.*, 1929, **6**, 839).

The constituents so far identified in the seeds as well as those observed in the preliminary investigations carried out by us some years ago, do not seem to account for their definite anthelmintic action. The report that the fresh seeds show much stronger anthelminitic action than the old, worm-eaten ones, coupled with the fact that the proteolytic enzyme present in the

milky juice of *Ficus laurifolia* has been proved to be the anthelmintic principle of the juice (Robbins, *J. Biol. Chem.*, 1930, **87**, 251) led us to look for enzymes in the fresh seeds. The literature does not show any reference to the presence of any enzyme in the seeds of *Butea frondosa*. The present investigation confirmed the presence of both proteolytic and lipolytic enzymes in the seeds and an account of the proteolytic enzymes forms the subject of this communication.

EXPERMENTAL.

For a preliminary examination, the following extracts were first made :

(1) 10% NaCl *Extract.*—Powdered seeds were soaked in the salt solution, thoroughly macerated. and the extract pressed out in a tincture press.

(2) 1% NaCl *Extract.*—Powdered seeds were treated with salt solution and the extract squeezed out.

(3) *Glycerine Extract.*—Powdered seeds were treated with 50% glycerine and the extract squeezed out in a tincture press.

(4) *Aqueous Extract* (enzyme cream).—5 G. of the powdered seeds were treated with distilled water (30 c.c.) and rubbed in a mortar for some time. The extract was squeezed out and the residue was again treated with water (30 c.c.) as before ; the total extract measured 50 c.c., 10 c.c. being equivalent to 1 g. of seeds.

A preliminary examination with the above extracts showed that the aqueous extract (4) was best suited for the study of proteolytic activity and all the experiments shown below were carried out with this extract. In all the experiments the formol titration method was used and the results are expressed in terms of $N/10$-caustic soda. Toluene was used as preservative in all the experimenrs.

TABLE I.

Digestion of Witte's Peptone without Buffer.

Substrate = 15 c.c. peptone (5% soln. in 1% saline). p_H adjusted with standard HCl or NaOH. Enzyme cream = 15 c.c. Temperature = 37°.

p_H	4·7	5·1	6·2	6·4	8·2
Hydrolysis after 21 hrs. (in c.c. $N/10$-NaOH)	0·6	1·1	1·4	1·4	2·7

The above results show that the enzyme acts better on the alkaline side.

TABLE II.

Digestion of Witte's Peptone with Buffer.

Substrate = 10 c.c. peptone (5% soln.). Phosphate buffer = 5 c.c. Enzyme cream = 10 c.c. Temperature = 37°.

p_H	4·3	5·5	5·8	6·5	7·5	8·5
Hydrolysis after 48 hrs. (in c.c. *N*/10-NaOH)	3·2	4·0	3·9	4·4	5·3	4·5

The results show that maximum hydrolysis takes place when the medium is just alkaline.

TABLE III.

Digestion of Casein.

The casein solution was prepared by rubbing in a mortar 4g. of Merck's extra pure casein with 30 c. c. of *N*/10-NaOH, diluting with water (120 c.c.) and filtering through cotton wool. Substrate = 10 c. c. casein solution. Phosphate buffer = 5 c. c. Enzyme cream = 10 c. c. Temperature = 37°.

p_H	5·0	6·0	7·0	8·0
Hydrolysis after 18 hrs. (in c.c. *N*/10-NaOH)	0·2	0·5	0·7	0·7

The results show that casein is very little acted upon. It is in accordance with the previous observation that plant erepsins are not usually capable of hydrolysing casein like animal erepsins.

TABLE IV.

Digestion of Egg-albumin.

Substrate = 15 c. c. egg-albumin (2% soln.). p_H adjusted with standard HCl or NaOH. Enzyme cream = 10 c. c. Temperature = 37°.

p_H	2·4	3·9	4·8	5·2	6·3	8·9
Hydrolysis after 21 hrs. (in c.c. *N*/10-NaOH)	0·0	0·0	0·2	0·3	0·4	0·9

There seems to be practically no action upon egg-albumin.

TABLE V.

Digestion of Gelatin.

2 C. c. of 5% gelatin containing 0·1% thymol were used as substrate. Different quantities of enzyme cream were added and the total volume made up to 4 c. c. by the addition of water; incubation for 24 hours at 37°.

Enzyme cream	Remarks.
2·0 c. c.	Liquefaction.
1·0	,,
0·5	,,
0·3	,,
Nil	Solidify on cooling.

TABLE VI.

Digestion of Fibrin.

A small flock of Merck's fibrin from blood stained with Congo-red was used as substrate. 1 C. c. of different extractives of butea seeds was added in each experiment; incubation for 24 hours at 37°.

Extractives of :—	Remarks.
10% NaCl	Negative.
Glycerine with 0·1 c. c. of *N*/10-HCl	,,
Glycerine with 0·1 c. c. of *N*/10-NaOH	,,
Water with 0·1 c. c. of *N*/10-HCl	,,
Water with 0·1 c. c. of *N*/10-NaOH	,,

No digestion of fibrin was observed; it probably pointed to the ereptic nature of the enzyme.

TABLE VII.

Study of Optimum Reaction of the Medium with different Substrates.

Substrate = 10 c. c. (3% soln.). Phosphate buffer = 5 c. c. Enzyme cream = 10 c. c. Incubation for 18 hours at 37°.

	Hydrolysis in c. c. of *N*/10-NaOH			
p_H.	Peptone	Casein	Egg-albumin.	Gelatin.
5·0	0·8	0·2	Very slight	0·0
6·0	0·7	0·5	,,	0·0
7·0	1·0	0·7	,,	0·0
8·0	1·2	0·7	,,	0·0

The results show that the enzyme acts best in the alkaline side, the optimum p_H lying in the neighbourhood of 8. It also shows that it acts best on peptone, there is some action on casein, while there is practically no action on egg-albumin and gelatin.

TABLE VIII.

Study of Optimum Temperature.

Substrate = 10 c. c. Phosphate buffer = 2 c. c. Enzyme cream = 10 c. c. Temperature = 37°. p_H of the medium = 8·0. Incubation for 4 hours.

	Hydrolysis in c. c. of *N*/10-NaOH				
Substrate.	at 30°.	at 40°.	at 50°.	at 60°.	at 70°.
Peptone	0·3	1·5	1·0	0·0	0·0
Casein	0·1	0·3	0·2	0·0	0·0

The results show that the optimum temperature is near about 40°.

TABLE IX.

Study of Activation of the Enzyme.

Substrate = 10 c. c. Phosphate buffer = 5 c. c. of p_H 7. Enzyme cream = 10 c. c. Activator = 1 c. c. Incubation for 20 hours at 37°.

	Hydrolysis (N/10-NaOH)	
Aetivator.	Peptone.	Casein.
—	1·0 c. c.	0·6
2% HCN	1·8	1·6
H_2S (sat. soln.)	0·6	0·6

The results show that HCN activates both in peptone and casein hydrolysis but H_2S is without action.

TABLE X.

Study of the Activation of Enzyme without any Buffer.

Substrate = 10 c. c. peptone. Enzyme cream = 10 c. c. Activator = 1 c. c. Incubation for 24 hours at 37°.

Activator.	p_H.	Hydrolysis in N/10-NaOH.
—	5·9	1·4 c. c.
2% HCN	5·5	2·1
H_2S (Sat. soln.)	5·7	1·6

The results show that there is definite activation with HCN but practically none with H_2S. The phosphate buffer does not seem to have any retarding action as observed in the case of some plant protease (Basu and Nath, *J. Indian Chem. Soc., Ray Com. Vol.*, 1933, p. 107 ; *ibid.*, 1936, **18**, 34).

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STUDIES ON THE ENZYMES OF THE SEEDS OF *BUTEA FRONDOSA*. PART II. LIPOLYTIC ENZYME.

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The fresh seeds of *Butea frondosa* were found to contain a lipolytic enzyme in addition to the proteolytic enzyme reported earlier. Its action has been studied in substrates like olive oil, castor oil and rape seed oil and it acts on all of these equally well. The lipase seems to act best in a slightly alkaline medium and shows an optimum temperature of about 40°. The aqueous emulsions of the raw seeds as well as that of the defatted seeds act equally well.

In the previous part of this paper (This Journal, p. 101) it was mentioned that a proteolytic enzyme and a lipolytic enzyme have been observed by us in the seeds of *Butea frondosa* and that there is no mention about these in the seeds in the literature. In studying the details of the lipase action, we followed mainly the methods of Willstätter and Waldschmidt—Leitz used in the study of *Ricinus lipase* (*Z. physiol. Chem.*, 1924, **134**, 161).

Experimental.

Preparation of Defatted Seeds.—The finely powdered seeds were treated with six times the weight of carbon tetrachloride and vigorously shaken in a flask for some time. The solid material was then filtered by suction and washed first with carbon tetrachloride and then with ether. It was then dried in a vacuum desiccator and stored in a bottle.

Preliminary Experiments with Defatted Seeds.—Olive oil (2·5 g.), defatted seeds (2·5 g.), phosphate buffer of p_H 7 (2 c.c.) were shaken vigorously in a flask for 3 minutes and then kept in an incubator at 37° for 20 hours. The mixture was then treated with an excess of alcohol and ether to stop the lipase action and titrated with $N/10$-caustic potash using phenolphthalein as indicator. A control experiment was also carried out without the use of the seed powder. The results showed strong lipolytic action but as they were not uniform the experiments were repeated with the use of an enzyme extract prepared as shown below.

Preparation of Aqueous Emulsion of Enzyme.—This was always prepared fresh by treating defatted seeds (10 g.) with distilled water (60 c.c.) added in

small portions and rubbed intimately in a mortar. The cream was pressed out through muslin and the residue again treated in the same manner with 60 c. c. of distiiled water and pressed out. The total extract amounted to 100 c. c.

Method of Estimation.—The substrate oil (2·5 g.) was weighed out in a small conical flask and mixed with the enzyme extract equivalent to 1 g. of the defatted seeds with the addition of 2 c. c. of a phosphate buffer. The contents of the flask were throughly mixed, kept in a thermostat at 37° for several hours. The mixture was then treated with 95% alcohol (30 c. c.) and ether (15 c. c.) and titrated with $N/10$-alcoholic potash using phenolphthalein as indicator. As the colour of phenolphthalein was rather quickly discharged, the end-point was noted immediately when the colour of the mixture became distinctly red. A control experiment without the enzyme extract was done in every case. The difference in the amount of $N/10$-alcoholic potash required gave a measure of the lipase action. The percentage of saponification was calculated (not shown) according to the following formula of Willstätter and Waldschmidt-Leitz:—

$$\text{Percentage of saponification} = \frac{V \times 56{\cdot}1 \times 100}{W \times S}.$$

where V is the volume of N-caustic potash; W the weight and S the saponification value of the oil taken. In an actual experiment, where 10 c. c. of extract, equivalent to 1g. of defatted seeds, was allowed to act for 20 hours at 37° on 2·5 g. of olive oil in the presence of phosphate buffer of p_H 8, the alkali required for the main experiment was 12·5 c.c. and that for the control 5·7 c. c. of $N/10$-caustic potash.

$$\text{Hence, percentage of saponification} = \frac{0{\cdot}68 \times 56{\cdot}1 \times 10}{2{\cdot}5 \times 190} = 8{\cdot}9\%.$$

In the Tables I—IV, 10 c. c. of aqueous emulsion of seeds (unless otherwise mentioned) equivalent to 1g. of seed and phosphate buffer (2 c. c.) have been used at a temperature, of 37°. Hydrolysis has been expressed in terms of c. c. of $N/10$-KOH.

TABLE I.

Substrate = 2·5 g. of olive oil (raw seed).

p_H	5·0	6·0	7·0	8·0
Hydrolysis after 20 hours	4·8	5·1	6·4	6·8

TABLE II.

Substrate = 2·5 g. of olive oil.

p_H	5·0	6·0	7·0	8·0
Hydrolysis after 20 hrs	4·9	5·9	6·1	7·2

TABLE III.

Substrate = 2·5 g. of castor oil.

p_H	5·0	6·0	7·0	8·0
Hydrolysis after 20 hrs.	5·4	5·4	6·2	7·0

TABLE IV.

Substrate = 2·5 g. of rape seed oil.

p_H	5·0	6·0	7·0	8·9
Hydrolysis after 20 hrs.	4·7	4·8	5·7	6·4

The results in the above tables show that the optimum reaction of the medium is a p_H of about 8.

TABLE V.

Study of Optimum Temperature.

Substrate = 2·5 g. of olive oil. 10 C. c. of aqueous emulsion of the seed equivalent to 1 g. of defatted seeds. Phosphate buffer at p_H 8. Incubation period 3 hours.

Temerature ...	30°	40°	50°	60°	70°
Hydrolysis ...	1·8	2·9	1·6	0·7	0·3

The maximum hydrolysis seems to take place at 40°; the reaction of the mixture being at its optimum p_H 8.

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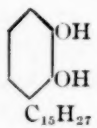
CHEMICAL EXAMINATION OF MANGO " CHEP", THE EXUDATION OF THE FRUIT OF *MANGIFERA INDICA*.

By Shiam Kishore Vasistha and Salimuzzaman Siddiqui.

The fresh mango " chep " in liquid condition as well as the semisolid mango chep resin have been investigated. The odorescent principle present in the fresh chep could not be isolated by steam distillation which appears to destroy it. From the dry mango chep a resen (mangiferen) $C_{21}H_{34}O$ a resinous acid (mangiferic acid) $C_{46}H_{60}O_4$, and a resinol (mangiferol) $(C_{21}H_{36}O_2)_5$, have been isolated and studied. Contrary to expectations, these resinous principles are found to be not allied to bhilawanol or anacardic acid. The degradation and oxidation products of mangiferen, which has been more fully investigated largely bring out their close relationship to the abietic acid series of resins, and supported the recent view of resins being condensation products of isopren

When the mango fruit is detached from its stem, a thin fluid exudes from it, which resembles terpentine in odour and consistency and soon dries up to a straw coloured, translucent, semi-solid mass which slowly loses its odorescent character. It is known in vernacular as 'Ám kí Chép' and is popularly regarded as a cure for scabies and other cutaneous affections. This reputed medicinal property, along with the fact that in its fluid condition, the mango ' chep ' is known to have vesicating properties, suggested to us the possibility of its chemical constituents being similar in composition to the various resinous principles obtained from the Anacardiacae family of plants (to which mango belongs), which have vesicating properties and are considered in indigenous medicine as cures for skin affections. Two of the cases in point are the juice of the pericarp of the 'marking nut' (*Semecarpus anacardium*, Linn.) which was found by Pillay and Siddiqui (*J. Indian Chem. Soc.*, 1931, **8**, 667) to consist chiefly of a vesicating principle, bhilawanol, whose formula was established by them as and



the juice from the pericarp of cashew nut (*Anacardium occidentale*, Lin.) consisting of anacardic acid as one of its constituents, which was found by Pillay (*J. Indian Chem. Soc.*, 1935, **12**, 226) to differ from bhilawanol only in the substitution of one of its hydroxyl groups by a carboxyl. It, therefore, appeared of great interest to us to investigate, if the mango 'chep' also

contained a similar resinous principle with a long, unsaturated, normal side-chain, attached to a catechol or salicylic acid nucleus.

It may be noted here that mango gum resin from the bark is stated by Hooper (*J. Pharm.*, 1907 *iv*, **24**, 718) to contain 79% of resin. According to Lemeland (*Pharm. J. Chem.*, 1904, *vi*, **19**, 584) the gum resin contains 60% water-insoluble and 40% water-soluble matter. No work, however, had so far been done on the mango 'chep' resin.

As a result of our investigation we have isolated from the dry chep the following three products, through a long drawn out process of separation, ensuring the standard of uniformity which may be acceptable in the chemical characterisation of resinous products.

(1) A resen, $C_{21}H_{34}O$, which distils constantly to a straw coloured brittle mass at 308-10°/5 mm., begins to soften at 60° and melts at 63-65°. $[\alpha]_D^{30} = +60°$ in 1% absolute alcoholic solution, yield 16% on the weight of the ether extract of the mango chep, and 4·5% of the semi-solid chep. We have given it the name "mangiferen".

(2) An acid, $C_{40}H_{60}O_4$, which melted slowly at 68-70°, $[\alpha]_D^{30} = +32°$ in 1% alcoholic solution which we have named "mangiferic acid", yield 2% of the ethereal extract.

(3) A phenol $(C_{21}H_{36}O_2)_5$, which gives greenish violet colouration with ferric chloride and a white precipitate with lead acetate and to which we have given the name "mangiferol," yield 3%.

Although the analytical values of the products noted above further strengthened the expectation of their being closely related to the products isolated from *Semecarpus anacardium*, a closer study of their chemical character, particularly of the chief constituent mangiferen, which was more fully investigated, definitely negatived this view.

Mangiferen gave negative results when tested for active H after the method of Zerewitinoff and formed no benzoyl, acetyl or naphthyl*iso*cyante derivatives. No methoxy group was found to be present after the method of Zeisel. It was left unaffected by caustic potash fusion at 220° and by concentrated sulphuric acid and also concentrated nitric acid (*d* 1·4). Its extreme indifference to chemical reagents as brought out by these observations clearly goes to show that mangiferen is a resen.

In so far as resens are known to be chemically indifferent products and their uniformity in most of the cases mentioned in literature is rightly considered doubtful*, the following observations made by us on mangiferen will appear to be of special interest.

* This fact has been particularly well brought out in a recent communication on the resen of the Manila-Elemi resin by Bauer and Starcke (*Arch. pharm.*, 1934, **272**, 167).

1. Mangiferen gives an oxidation product, $C_{21}H_{34}O_2$, when treated with a large excess of potassium permanganate (in acetone solution).

2. On dry distillation and subsequent fractionation of the distillate over sodium, two hydrocarbons answering to the molecular formulæ $C_{15}H_{26}$ and $C_{15}H_{24}$ were obtained.

This point is of special significance in so far as resens have more recently been variously indicated as isoprene condensation products (Wagner-Jauregg, *Annalen*, 1932, **496**, 52 ; Bauer and Umbach, *Ber.*, 1916, **49**, 794).

3. On treatment with fuming nitric acid, it yielded an acid which was not sufficient in quantity for more complete investigation but whose analysis indicated that it might be a saturated dicarboxylic acid, $C_8H_{12}O_4$, like the racemic *trans*-hexahydrophthalic acid, obtained by Levy (*Z. anorg. Chem.*, 1913, **81**, 150,) through a similar oxidation of abeitic acid.

The water-soluble portion of dry mango chep consisted of gums, etc., and contained no tannic or gallic acids.

Fresh mango chep was worked on the same lines as detailed in the case of dry chep, and was found to yield similar fractions. In contrast to the dry chep, the ether extract of the fresh chep had the characteristic odour of the freshly detached mango, but no essential oil could be obtained from it through steam distillation which apparently destroyed the odorescent principle.

EXPERIMENTAL.

Mango chep (500 g.) was collected during the mango season from several large consignments of mangoes, through special arrangements made by Hakim Zafar Khan Sahib. We take this opportuuity to express our thanks for the pains he took in this connection, without which it would not have been possible to carry out the present investigation.

Separation of the Constituents of "Mango Chep."

The mango chep thus collected (500 g.) formed a semi-solid sticky mass. It was divided into water-soluble and ether-soluble fractions. The ether-soluble part (150 g.) was then taken up in alcohol and treated with an excess of alcoholic lead acetate solution. The filtrate was made ammoniacal, allowed to settle and filtered from the lead salt. The clear filtrate was deleaded with hydrogen sulphide and the residue left on removal of the solvent was taken up in petroleum ether and treated with caustic soda, when a small quantity of a black sticky matter settled down. The straw

coloured filtrate was washed with dilute hydrochloric acid and finally with water, was dried over sodium sulphate and freed of the solvent, when it yielded 25 g. of an extremely sticky straw coloured product. (Found : C, 82·9 ; H, 11·2 per cent).

This neutral matter (1 g.) was fractionally distilled under reduced pressure and the middle fraction was analysed. (Found : C, 83·0 ; H, 11·7 per cent). Having thus ascertained that no intrinsic change takes place by distillation in *vacuo* the remaining quantity (24 g.) was subjected to fractional distillation in *vacuo* whereby four fractions were separated. The third and main fraction was redistilled, when *mangiferen* was obtained as a straw coloured product (20 g.), distilling at 308-12°/5 mm. (main quantity at 310°).

The lead salt from the ether extract of the chep was exhaustively extracted with ether. The ethereal solution was well shaken up with hydrochloric acid to remove the lead, washed well with water, dried over sodium sulphate and freed of the solvent. For complete removal of any neutral matter from this residue, it was once again purified through the lead salt as described above, and then taken up in ether. The ethereal solution was treated with 5% caustic soda solution in a separating funnel, when a pink coloured granular middle layer sharply isolated itself from the ether layer above and the alkaline solution below. After repeatedly washing this layer with ether and 5% caustic soda solution, it was acidified with dilute hydrochloric acid in the presence of ether, the clear ethereal layer was washed with water, dried over sodium sulphate and freed of the solvent. The sticky residue was then repeatedly washed with boiling petroleum ether when it was reduced to a buff coloured light powder (14 g.). By a series of fractional precipitations with benzene and petroleum ether, 3 g. of *mangiferic acid* was finally obtained as the petroleum ether-insoluble and benzene-soluble fraction.

The petroleum ether-soluble, acidic fractions from the above were taken up in ether and exhausted with 0·5% caustic soda solution. The pink, alkaline solution was repeatedly washed with ether. It was then freed of dessolved ether on the water-bath, filtered after cooling, acidified with hydrochloric acid and extracted with ether. The ethereal layer was washed with water, dried and freed of the solvent. The residue (about 12 g.) was once again purified through the lead salt when *mangiferol* was finally obtained as a reddish brown, petroleum ether-soluble treacle (5 g.). Several intermediate fractions, apparently mixtures of mangiferic acid and mangiferol, were not pursued further.

Characterisation of the Constituents.

Mangiferen ($C_{21}H_{34}O$).—As obtained by distillation it forms a straw coloured brittle mass. On precipitation of this product from its alcoholic solution with water it forms a white powder. Both these samples show the same optical activity $[\alpha]_D^{30} = +60°$ in 1% absolute alcoholic solution. It begins to soften at 60°, melts at 63-65° and is soluble in all common organic solvents from which, however, mangiferen could not be induced to crystallise. [Found : C, 82·9, 82·9, 83·1, 83·7 ; H, 11·0, 11·2, 11·6, 11·7 ; mean. C, 83·2 ; H, 11·4 ; M. W. (cryoscopic in benzene), 336. $C_{21}H_{34}O$ requires C, 83·4 ; H, 11·3% ; M. W. 302 ; $C_{21}H_{32}O$ requires C, 84·0 ; H, 10·6%). It gave no colour with ferric chloride or precipitate with basic lead acetate. It contained no hydroxyl group as was shown firstly by the negative result of active hydrogen estimation after Zerewitinoff and secondly by its failure to form an acetyl or a naphthylurethane derivative. Methoxy group also was found to be absent as tested after Zeisel. After stocking for a year, mangiferen showed the same optical activity, but it began to soften at 63° and melted completely only at 90°. Mangiferen dissolves to a deep golden yellow solution in concentrated sulphuric acid, which later turns orange. On dilution the original product is recovered from this solution even after heating it on the water-bath for some time. On addition of a few drops of concentrated sulphuric acid to a solution of mangiferen in acetic anhydride a blood red colouration is produced, which darkens on standing taking on a brownish yellow tinge. Fusion with caustic potash at 220° yielded back the original product unaffected.

Oxidation of Mangiferen with Potassium Permanganate : Oxymangiferen ($C_{21}H_{34}O_2$).—To mangiferen (2 g.) dissolved in acetone (200 c.c.) and water (30 c.c.) was added slowly with stirring and occasiona warming on the water-bath, potassium permanganate (7 g.) dissolved in acetone (200 c.c.) and water (30 c.c.). On working up the reaction product no acid could be separated, most of the substance having remained unreacted. This was subsequently treated with twice the above quantity of potassium permanganate in the same concentration. The reaction mixture was filtered and the filtrate freed from acetone on the water-bath. The residue was treated with dilute alkali and extracted with ether. The alkali-soluble portion did not yield any appreciable acid fraction on acidification. The ethereal extract was washed with dilute alkali, then acid and finally with water, dried over sodium sulphate and freed of the solvent. The treacly residue was exhausted with petroleum ether to remove the unreacted neutral matter. The petroleum ether-insoluble residue was obtained as a

colourless light powder on complete removal of the solvent in *vacuo*. Oxymangiferen thus obtained, melted at 85°. Like mangiferen it gave no colouration with ferric chloride and no precipitate with basic lead acetate. (Found: C, 79·3; H, 10·3. $C_{21}H_{34}O_2$ requires C, 79·2; H, 10·7 per cent. $C_{21}H_{32}O_2$ requires C, 79·8; H, 10·1 per cent). Oxymangiferen appears to have been formed through the subsequent splitting of a molecule thus of water from the initial product ($C_{21}H_{36}O_3$) of the permanganate oxidation of mangiferen.

Dry Distillation of Mangiferen.

Mangiferen (5 g.) was heated at ordinary pressure when it distilled to a reddish brown jelly like substance contaminated with a few drops of water. On repeated fractional distillation of this distillate an oily liquid (0·5 g) distilling at 218-21° separated. (Found: C, 87·4, H, 9·9 per cent). Another fraction (about 0·5 g.) distilling at 278-80° was of a thicker consistency. (Found: C, 88·0; H, 10·4 per cent). As an oxygen-free substance at this stage could not be isolated, the various distillates were combined together and distilled over sodium and the distillate again fractionally distilled, when two main fractions distilling at 225-35° and 260-70° and showing $n_D^{35} = 1{\cdot}4970$, and 1·5155 respectively were obtained. 0·1010 G. of the lower boiling fraction took up 0·1386 g. of bromine in chloroform solution in cold as against 0·1581 g. required by $C_{15}H_{26}$ for two double bonds. (Found: C, 86·7; H, 13·2. $C_{15}H_{26}$ requires C, 87·4; H, 12 6 per cent). The higher boiling fraction gave C, 86·8; H, 12·3. $C_{15}H_{24}$ requires C, 88 2; H, 11·8 per cent).

Oxidation of Mangiferen with Fuming Nitric Acid.

Concentrated nitric acid having failed to act on it even on heating, 2 g. of mangiferen were dissolved in glacial acetic acid (20 c.c.) and to the solution (20 c.c.) fuming nitric acid was slowly added with ice cooling and stirring. The reaction mixture was placed on the water-bath for about 30 minutes, taking care that the reaction did not become too vigorous, and then added to excess of cold water. A cream yellow acid separated out which was washed free of nitric acid It was soluble in dilute alkali, alcohol, ethyl acetate, glacial acetic acid, partly soluble in ether and practically insoluble in petroleum ether and benzene. It could not be crystallised but by purification through alcohol and water its melting point could be raised from 175° to

188° (decomp.). [Found : C, 56·2; H, 6·3; M.W. (cryoscopic in glacial acetic acid), 185. $C_8H_{12}O_4$ requires C, 55·8; H, 7·0 per cent. M.W., 172].

A small quantity of a yellowish semicrystalline powder completely insoluble in benzene also separated from the reaction product, which was insoluble in petroleum ether but soluble in ethyl acetate and melted at 218° (decomp.) ; racemic *trans*-hexahydrophthalic acid melts at 218°.

Addition of Bromine.

Mangiferen (0·1013 g.) was titrated against bromine in chloroform solution with ice cooling. Following the titration with potassium iodide starch paper, it took 0·0577 g. of bromine as against 0·0537 g. required for one double bond.

Mangiferic Acid ($C_{40}H_{60}O_4$).

It is a colourless light powder, soluble in benzene, ether, alcohol and insoluble in petroleum ether, m.p. 76-78°. It forms a water-insoluble sodium salt, a gelatinous ammonium salt and ether-soluble lead salt with basic lead acetate. It shows $[\alpha]_D^{30} = +32°$ in 1% absolute alcoholic solution. The air dried product loses no weight on heating at 110°. In concentrated sulphuric acid it dissolves to a golden yellow solution. [Found : C, 79·0; H, 10·4; M.W. (cryoscopic in benzene), 685. $C_{40}H_{60}O_4$ requires C, 79·5; H, 9·9 per cent. M.W., 604). Of the two formulae, $C_{40}H_{60}O_4$ and $C_{50}H_{75}O_5$, possible on the basis of the M.W. value, preference has been provisionally given to the former, as parallel M.W. determinations by us, for allied resinous products, showed the error to be invariably on the positive side. Methoxy group was found to be absent in mangiferic acid after Zeisel's method.

On treatment with acetic anhydride and a few drops of concentrated sulphuric acid, mangiferic acid gave a deep red colouration and yielded on dilution a light brown semicrystalline powder, which lost moisture from 108-135°, slowly decomposed from 230° onwards, and appeared to be a diacetyl derivative from its analysis. [Found : C, 76·7; H, 9·8. Diacetyl mangiferic acid ($C_{44}H_{64}O_6$) requires C, 76·7; H, 9·3 per cent. Monoacetyl mangiferic acid ($C_{42}H_{62}O_5$) requires C, 78·0; H, 9·6 per cent].

0·103 G. of mangiferic acid in chloroform solution took 0·0554 g. of bromine in the cold as agaiust 0·0546 g. required for 2 double bonds. The absorption near the end of the titration was comparatively very slow.

Mangiferol $(C_{21}H_{36}O_2)_5$.

Mangiferol is a sticky, reddish brown product, readily soluble in ether, petroleum ether and benzene, less so in alcohol. It forms a water-soluble sodium salt and an ether-soluble lead salt. It gives phenolic colouration with ferric chloride, but can not be acetylated by the usual method. It absorbs bromine in the cold but can not be hydrogenated in the presence of platinum black. [Found : C, 78·8; H, 11·4 ; M. W. (cryscopic in benzene), 1632. $(C_{21}H_{36}O_2)_5$ requires C, 78·8 ; H, 11·3 per cent. M.W., 1600]. The lead salt, prepared by adding alcoholic lead acetate solution to the alcoholic solution of mangiferol, gave after drying in *vacuo* Pb, 24·3. $(C_{42}H_{70}O_4Pb)_5$ requires Pb, 24·5 per cent]. Methoxy group was found to be absent after Zeisel's method.

One of the authors (S. K. V.) is highly indebted to the Benares Hindu University for granting him leave which enabled him to take part in the present investigation. We also take this opportunity to thank Chaudhuri Ishratali for making arrangements for collecting fresh mango cheap from his gardens near Lucknow.

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A NOTE ON THE ACTION OF THE CHLORINE-BLEACH ON THE RESIN CONSTITUENTS OF LAC.

By M. N. Murty

A study of the chemical changes undergone by the resin constituents of lac during bleaching with hypochlorite solution has been made.

Bleaching is accompanied by an increase in the acid value and an apparent increase in the saponification value and ester value of lac. On making due allowance for the reaction between the chlorine present in bleached lac and the alcoholic potash used in saponification, real saponification and ester values can be calculated. The real (or corrected) saponification value of shellac is about 5% higher than that of unbleached lac, thus indicating an increase in the carboxyl groups due to oxidative changes brought about by the chlorine bleach. Aleuritic acid, a constituent of lac, does not appear to have been affected by the chlorine-bleach. The hydroxyl number generally decreases slightly on bleaching.

Chlorine enters into the resin molecules, and its content increases steadily as the lac is progressively bleached, while the iodine value decreases rapidly in the beginning and then tends to attain a minimum value. The chlorine in bleached lac exists only partly as an addition compound the excess entering the molecule through substitution. The chemical changes, *viz.*, oxidation and chlorination, are brought about in the resin molecules almost immediately after the addition of the bleaching agent. But the changes in the colouring matter, although proceeding simultaneously, take some time to attain completion. The rate of absorption of bleach is quicker in poor bleaching lacs than in good Kusum lac and is further enhanced by employing a higher temperature and lower p_H .

When bleached lac is allowed to remain in the alkaline solution after all the added hypochlorite has been consumed, the following changes take place, the extent of these changes depending upon the duration, temperature and p_H.

(*a*) The chlorine content decreases, the unsatuaration increases due to splitting off of hydrocholoric acid and the colour of the bleached lac tends to return.

(*b*) The acid value increases due to hydrolysis, consequently the ester value decreases.

(*c*) The saponification value appears to decrease slightly due probably to the splitting off of carbon dioxide from the carboxyl groups or due to removal of some acid constituent of lower molecular weight during washing.

A slower rate of attack on the resin molecules by a gradual addition of the bleaching liquor gives a product which is less prone to become insoluble than a rapidly bleached product. Higher alkalinity of the bleaching liquor and other conditions which bring about hydrolysis give a bleached lac which will have a longer life, but which will have the disadvantage of high acid value and poor colour.

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REVIEW

Ebulliometry—By Professor W. Swietosawski—published by the Jagellonian University Press—pp. (X + 196).

Professor Swietosawski and his assistants at the Polytechnic of Warsaw have been working at the most exact measurements of boiling points and vapour pressures of liquids and solutions for the last 15 years or more, and they have succeeded in improving the ebulliometric and tonometric instruments and devices to such an extent as to make possible exact determination of temperature and pressure to the thousandth of a degree and of a mm. respectively. The book under review describes the modifications and improvements of the ebulliometric apparatus of Washburn and Cottrell and the importance and utility of such exact measurements. The test for the purity of liquid substances, relation between compositions of vapour and binary liquid mixture, and the behaviour of homoazeotropic and heteroazeotropic liquid mixtures under boiling conditions have been discussed in detail. The sixth chapter which describes the behaviour of azeotropic mixtures should have been provided with results of actual measurements in tabular form and not merely qualitative figures with page-long descriptions which make the thing tedious-reading. The accuracy attempted at is really astonishing and the methods described will be of help to those investigators who want to work with tested super-pure liquids.

H. L. R.

SYNTHESIS IN THE ALLOXAZINE, *ISO*ALLOXAZINE (FLAVIN) AND LUMAZINE GROUPS. PART III. SYNTHESIS OF SOME ACID DERIVATIVES.

By K. Ganapati.

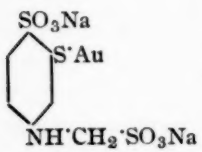
The synthesis of some acids of the alloxazine and flavine groups has been undertaken for some chemotherapeutic purposes. *o*-Phenylenediamine-4-sulphonic acid and 1 :2-diaminonaphthalene-4-sulphonic acid yield with alloxan the corresponding alloxazine derivatives. Flavin-9-propionic acid has been synthesised starting from *o*-nitroaniline by the method of Kuhn, but attempts to synthesise the corresponding 6-methyl derivative did not meet with success, a product, m.p. 225°, being obtained instead. 3 :4 Diaminocinnamic acid yields with alloxan the corresponding alloxazine-acrylic acid.

As has been pointed out in Part I (*J. Indian Chem. Soc.*, 1937, **14**, 627) one of the main objects of the synthesis in this series is to prepare compounds that may be least toxic to the host and also possess some specific physical and pharmacological properties. The presence of the acid groupings in the case of numerous drugs is indeed striking and this is mainly due to the fact that these groupings, besides lowering the toxicity of the compounds, form soluble sodium salts which make their administration easier and absorption more effective. The synthesis of different types of acids of the above groups has, therefore, been attempted. Kuhn and Boulanger (*Z. physiol. Chem.*, 1936, **241**, 233) studied the lethal doses of many carboxylic acids of alloxazine and flavine groups on mice and rabbits and have found that the acid groupings decrease the toxicity of the compounds in many cases.

Kuhn and Cook (*Ber.*, 1937, **70**, 764) condensed 3:4-diaminobenzoic acid with alloxan and obtained the two theoretically possible isomeric acids, alloxazine-6- and 7-carboxylic acids. It appeared to us worth while to compare these with the corresponding sulphonic acid, since in many of the dyestuffs used in chemotherapy, the presence of the sulphonic acid grouping is known to decrease the toxicity of the compound and also to possess some specific property of facilitating the absorption of the drug (Stieglitz, " Chemistry and Recent Progress in Medicine", 1926, p. 12; Ehrlich, " Studies in Immunity", 1910, p. 414). It is of interest to note that of the two drugs, solganol (I) and krysolgan (II), used in the treatment of tuberculosis, the former is less toxic, better tolerated and generally gives better results (*cf.* Pai, *Indian Med. Gazette*, 1928, May supplement,

p. 18; Freund, *Beitr. Klin. Tuberk.*, 1928, **68**, 606; Schröder, *Z. Tuberk.*, 1934, **70**, 408).

o-Phenylenediamine hydrochloride was sulphonated and the resulting 1:2-diaminobenzene-4-sulphonic acid (Post, *Annalen*, 1880, **205**, 100) condensed with alloxan to yield (apparently) a mixture of acids from which an acid was isolated which can be represented as either alloxazine-6-sulphonic acid (III, R=H; R′=SO_3H) or alloxazine-7-sulphonic acid (III, R=SO_3H; R′=H). This acid is comparatively more soluble in water than other alloxazine derivatives. To prepare an acid of higher molecular weight, 1:2-diaminonaphthalene-4-sulphonic acid was condensed with alloxan and the product obtained is probably 7:8-benzoalloxazine-6- sulphonic acid (IV) or 5:6-benzoalloxazine-7-sulphonic atid (V) or a mixture of both.



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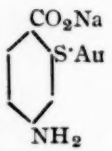

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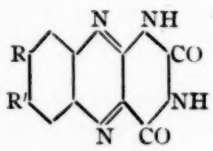

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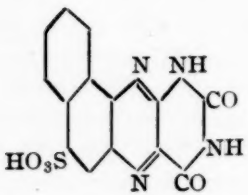

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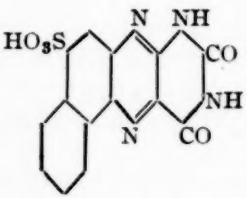
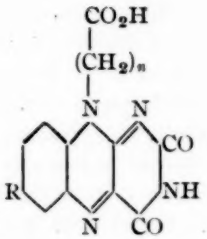


(V)

Though β-naphthoquinone has been found to condense with 4:5-diaminouracil sulphate to yield benzolumazine (Kuhn and Cook, *loc. cit.*), β-naphthoquinone-4-sulphonic acid did not, however, react with the uracil to yield either (IV) or (V). This is probably due to the fact that the sulphonic acid group in β-naphthoquinone-4-sulphonic acid has a great tendency to react first with the amino group and then pass on to the α-quinonoid form (*cf.* Boniger, *Ber.*, 1894, **27**, 25).

It has been observed by Kuhn and Boulanger (*loc. cit.*) that flavin-9-acetic acid (*iso*alloxazine-9-acetic acid, VI, R=H, n=1) is less toxic than the corresponding compound without the acid grouping. With an object to

find out the effect of lengthening the side-chain by one more carbon atom, in position 9, the synthesis of flavin-9-propionic acid (*iso*alloxazine-9-propionic acid, VI, R=H ; $n=2$) was undertaken. This compound has been synthesised very recently by Karrer, Köbner and Zehender (*Helv. Chim. Acta*, 1936, **19**, 261) starting from *o*-nitrobromobenzene. It has now been synthesised according to the method of Kuhn and Rudy (*Ber.*, 1935, **68**, 300) in better yield. *o*-Nitroaniline condensed with β-bromopropionic acid yielding *N*-(*o*- nitrophenyl)-β-aminopropionic acid (VII, R=H), which, after reduction with alkaline stannite and subsequent condensation with alloxan in acid solution, yielded flavin-9-propionic acid (VI, R=H; $n=2$).



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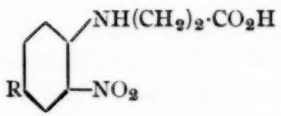

(VII)

In order to synthesise 6-methylflavin-9-propionic acid (VI, R=Me ; $n=2$), 4-methyl-2-nitroaniline was condensed with β-bromopropionic acid to yield *N*-(4-methyl-2-nitro)-β-aminopropionic acid (VII, R=Me), which after reduction with alkaline stannite and treatment with alloxan, however, did not yield the corresponding flavin derivative but only a product, m.p. 225°, which has not yet been identified and does not appear to be either the amino compound corresponding to VII (R=Me) or the lactam derived from it. It is indeed striking that the presence of the methyl group so radically alters the course of the reaction.

It appeared to be of interest to synthesise an acid with a side-chain attached to the benzene ring and 3:4-diaminocinnamic acid was condensed with alloxan. The product obtained can be either alloxazine-6-(β)-acrylic acid (III, R=H; R′=HO_2C·CH:CH-) or alloxazine-7-(β)-acrylic acid (III, R=HO_2C·CH=CH- ; R′=H) or a mixture of both. The unsaturated side chain is expected to impart to the compound some specific pharmacological properties, because cinnamic acid has been reported to give good results in the treatment of some forms of tuberculosis (Stenberg, *Z. Tuberk.*, 1927, **48**, 309 ; *cf.* also Jacobsen, *Res. prakt. Biol.*, 1929, **21**, 188).

EXPERIMENTAL.

Alloxazine 6- or 7-sulphonic Acid (III).—*o*-Phenylenediamine hydrochloride (2·5 g.) was carefully added to fuming sulphuric acid (30% SO_3, 18 c.c.) and the mixture heated on the water-bath for 4-5 hours. It was then poured into ice (about 300 g.), the dilute solution carefully neutralised with barium carbonate, filtered and the filtrate (charcoal) was concentrated on the water-bath to a small volume. The solid matter was filtered off after chilling, the filtrate made just acid with dilute sulphuric acid, the precipitated barium sulphate filtered off and the clear solution containing the sulphonic acid just warmed on the water-bath with a solution of alloxan (3 g. in 30 c.c. of water) for a few minutes, whereby the condensation product began to separate in fine yellow needles. It was filtered after allowing to stay for about an hour and then crystallised twice from boiling water. It separates in fine yellow needles which appear homogeneous. (Found: N, 18·88. $C_{10}H_6O_5N_4S$ requires N, 19·05 per cent). The substance, probably because of its nonfusible nature,* does not decompose completely when heated with concentrated nitric acid in the bomb so that the estimation of sulphur could not be carried out accurately. It is almost insoluble in all organic solvents and comparatively more soluble in boiling water. Its solution shows a green fluorescence.

The condensation product appears to yield a mixture of two products (as judged from the crystalline form) and the isomer, other than the one described, could not be readily isolated.

7:8-Benzoalloxazine-6-sulphonic Acid (IV), *5:6-Benzoalloxazine-7-sulphonic Acid* (V).— Congo red (2·3 g.) was dissolved in boiling water (50 c.c.) and the solution mixed with ammonia (25 c.c.). On the addition of excess of zinc dust and vigorous stirring, the colour of the solution was discharged and benzidine began to separate. After about ½ hour the solution was kept well cooled in ice for another ½ hour, the excess of zinc dust and benzidine carefully filtered off, the filtrate acidified with concentrated hydrochloric acid and warmed on the water-bath for a few minutes after the addition of a solution of alloxan (1·5 g.), when the yellow condensation product began to separate. After allowing to stand for about an hour, it was filtered and washed well with cold water and alcohol, yield 1·5 g. It separated from boiling water in orange-yellow amorphous form. (Found : N, 16·52. $C_{14}H_8O_4N_5S$ requires N, 16·28 per cent). It is very sparingly soluble in organic solvents

* The melting points of the compounds, wherever they are not definite, are not recorded.

and comparatively fairly soluble in boiling water from which it separates sometimes in a colloidal form very difficult to filter. It gives a cherry red colouration with concentrated sulphuric acid.

N-(o-*Nitrophenyl*)-*β-aminopropionic acid* (VII, R=H) has been prepared by Karrer, Köbner and Zehender (*loc. cit.*) by condensing *o*-nitrobromobenzene with *β*-aminopropionic ester and hydrolysing the product. It can be prepared more conveniently by the following method. An intimate mixture of *o*-nitroaniline (13·8 g.) and *β*-bromopropionic acid (15·3 g.) was kept at 140-50° for 1 hour (the yield is better at this temperature than at 110-20°). After cooling, the mass was triturated with dilute ammonia, filtered and the alkaline filtrate acidified under cooling with concentrated hydrochloric acid, whereby the condensation product separated. The mixture was chilled and then filtered, yield 6·5-7·5 g. It crystallised from dilute alcohol, m.p. 141-42°. (Found : N, 13·23. $C_9H_{10}O_4N_2$ requires N, 13·33 per cent).

The yield of the product in this case is far less than in the case of condensation product of *o*-nitroaniline with bromoacetic acid; it was thought that this was due to the *β*-position of the bromine atom. But it has been found (unpublished observation) that *β*-bromopropionic acid condenses with *m*-nitroaniline under the same conditions to give a good yield of *N*-(*m*-nitrophenyl)-*β*-amino propionic acid.

Flavin-9-(β)-propionicAcid or isoAlloxazine-9-propionic Acid (VI, R=H; *n*=2).—A solution of N-(*o*-nitrophenyl)-*β*-aminopropionic acid (2·2 g.) in sodium hydroxide solution (10 %, 20 c.c.) was added with stirring to a solution of alkaline stannite, prepared from a solution of stannous chloride (9 g. in 20 c.c. of water) and sodium hydroxide (10%, 90 c.c.). In about 10-15 minutes the red colour was discharged and the solution became light yellow indicating that the reduction was complete; the solution was added drop by drop with good stirring to alloxan (3 g.) in glacial acetic acid (75 c.c.) whereby a yellow compound separated. After about 15 minutes, the mixture was acidified with concentrated hydrochloric acid (15 c.c.) and after adding powdered sugar charcoal (40 g.) it was kept stirred for 1 hour when all the dyestuff was absorbed. The charcoal was filtered, washed thoroughly with warm and then cold water and the dyestuff eluted by means of pyridine-methanol-water mixture. On distilling off most of the solvent from the yellow solution showing a green flourescence in partial *vacuo* on the water-bath, the dyestuff separated. After allowing to stand for sometime, it was filtered, dissolved in dilute sodium hydroxide, filtered and the clear solution acidified with acetic acid whereby the dyestuff was thrown down. This was collected and washed well with water, acetone and

alcohol, yield 0·6 g. This was crystallised according to the directions of Karrer (*loc. cit.*). (Found : N, 19·36. $C_{13}H_{10}O_4N_4$ requires N, 19·58 per cent).

In some experiments, wherein the reason could not be exactly traced, instead of the flavin acid, a new yellow product was isolated. This differs from the flavin derivative in being fairly easily soluble in alcohol and acetone and showing no fluorescence in alcoholic or pyridine solution. It crystallises from dilute alcohol or pyridine in golden yellow needles, m.p. 219°. (Found : C, 49·56 ; H, 3·92 ; N, 15·52 per cent). It can also be obtained as a colourless product under a particular set of conditions. It dissolves in sodium bicarbonate without effervescence and the alkaline solution shows no fluorescence. The same product could not be obtained by reducing *N*-(*o*-nitrophenyl)-*β*-aminopropionic acid with either alkaline stannite or ferrous sulphate and ammonia and then acidifying with acetic acid. From the analytical figures with a high oxygen content, it appears to be a product of some complex reaction. It could not be further investigated owing to the small quantity of the material at the disposal.

N-(2-*Nitro*-4-*methylphenyl*)-*β*-*aminopropionic acid* (VII, R = Me) was prepared just as in the previous case by heating an intimate mixture of 2-nitro-4-methylaniline (15 g.) and *β*-bromopropionic acid (15 g.) at 145-50° for 1 hour, dissolving the product in dilute ammonia, filtering and acidifying the filtrate under cooling, yield 5·5-6·5 g. It crystallised from alcohol in shining red prisms, m. p. 148-49°. (Found: N, 12·48. $C_{10}H_{12}O_4N_2$ requires N, 12·50 per cent).

From the above compound the preparation of the flavin-propionic acid was attempted following the exact conditions as in the above case. Instead of the desired product, a compound crystallising in yellow needles or flakes, m.p. 225°, formed the main bulk of the product. This appears to resemble the product (m.p. 219°), described previously, in its properties. This could not be obtained by reducing the nitro compound with alkaline stannite, stannous chloride and hydrochloric acid or ferrous sulphate and ammonia. This is being further examined.

3-*Nitro*-4-*aminocinnamic Acid*.—*p*-Nitrobenzaldehyde (20 g.), reduced by a solution of sodium bisulphite (35%, 200 c. c.) essentially according to the method of Cohen and Springer (*Manatsh.*, 1903, **24**, 87), gave *p*-aminobenzaldehyde hydrochloride (28 g., Cohen and Springer get 20 g.) which was directly acetylated by boiling with acetic anhydride and sodium acetate. Even after repeated crystallisations *p*-acetaminobenzaldehyde was found to melt only at 153° (Cohen and Springer give m.p. 161°, while Gabriel and Herzberg, *Ber.*, 1883, **16**, 2003, give m.p. 153°). 3-Nitro-4-acetamino-

cinnamic acid can be prepared from it by the following two methods:

(*i*) *p*-Acetaminobenzaldehyde (12 g.), dissolved in concentrated sulphuric acid (100 c. c.) below 15°, was cooled to -5° and a mixture of nitric acid (*d* 1·51; 20 c. c.) and concentrated sulphuric acid (*d* 1·84 ; 30 c.c.) was slowly added with good stirring without appreciable rise in temperature. The mixture after keeping at this temperature for ½ hour was poured into ice, filtered and the nitrated product crystallised from water, yield of 3-nitro-4-acetaminobenzaldehyde was about 11 g.

A mixture of 3-nitro-4-acetaminobenzaldehyde (11 g.), malonic acid (12 g.), pyridine (15 c. c.) and piperidine (1 c. c.) was heated on the water-bath for 1 hour, then on the sand-bath for 15 miuutes and poured into dilute hydrochloric acid (250 c. c.), whereby 3-nitro-4-acetamino-cinnamic acid crystallised out in almost quantitative yield.

(*ii*) A mixture of *p*-acetaminobenzaldehyde (10 g.), malonic acid (11 g.), pyridine (20 c. c.) and piperidine (1 c. c.) was heated on the steam-bath for 1½ hours and on the sand-bath for 15 minutes and then mixed with dilute hydrochloric acid (200 c. c.), whereby *p*-acetaminocinnamic acid crystallised out (yield 9·5 g., some more of the product being obtained from the mother liquors on standing).

If nitrated with very concentrated acid at the room temperature or even at 0°, there is a pronounced tendency for the compound to get decarboxylated with the production of the styrene derivative. Gabriel and Herzberg (*loc. cit.*) nitrated it at -12° to -14° with fuming nitric acid. The following condition appears to be more satisfactory. *p*-Acetaminocinnamic acid (7·5 g.) was added with stirring to nitric acid (*d* 1·42; 50 c. c.) with good stirring, care being taken to keep the temperature between 15-20°. After all the product was added the mixture was carefully raised to 25° for a few minutes, poured into ice, filtered, the product dissolved in dilute sodium hydroxide, and the solution carefully acidified with concentrated hydrochloric acid when 3-nitro-4-acetaminocinnamic acid separated, yield 7 g.

Hydrolysis of this product to 3-nitro-4-aminocinnamic acid was carried out according to Gabriel and Herzberg (*loc. cit.*). After crystallising from water it melted at 225° (decomp.) (*cf.* lit. m.p. 224·5°; 218°).

Alloxazine 7- or 8-(*β*)-*acrylic Acid.*—3-Nitro-4-aminocinnamic acid (1 g.) dissolved in 2*N*-NaOH, was added to alkaline stannite solution from stannous chloride (3·5 g. in 25 c. c. of water) and 2*N*-NaOH (60 c. c.) and stirred well for ½ hour. To the light brown solution obtained was added with stirring a solution of alloxan (2 g. in 20 c. c. of water) and glacial acetic acid (50 c. c.), when the yellow condensation product separated. The solution was

acidified with concentrated hydrochloric acid (10 c.c.) and kept for 2 hours. Sugar charcoal (15 g.) was added to it and the solution was stirred for 1 hour whereby the yellow product was absorbed. The charcoal was filtered, throughly washed with water and the dyestuff eluted by means of pyridine-methanol-water mixture. The yellow solution showing a green fluorescence was concentrated to a small volume under reduced pressure when the yellow product separated, yield 1 g. It was obtained from boiling water as a yellowish orange spongy mass. (Found: N, 19·98. $C_{13}H_8O_4N_4$ requires N, 19·72 per cent.) In dilute alkaline solution it shows a green fluorescence and very easily decolourises alkaline permanganate.

The author thanks Dr. P. C. Guha for his keen interest in the work, Prof. J. N. Ray for a micro-analysis and the authorities of the Lady Tata Memorial Trust for the award of a scholarship which enabled him to continue his investigation.

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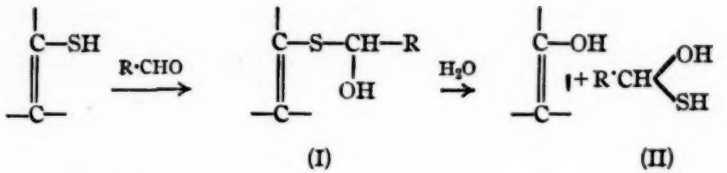
THIOKETONIC ESTERS. PART VI.

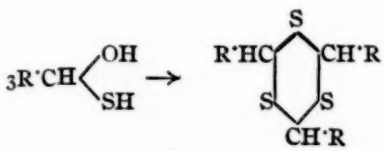
By S. K. Mitra.

The condensation of aldehydes with β-thioketonic esters has resulted in the formation of β-trithioaldehydes. A mechanism of the reaction has been suggested. The β-thioketonic esters react in the thiol phase.

The reaction of benzaldehyde with ethyl thioacetoacetate in the presence of acid condensing agent furnished β-trithiobenzaldehyde (Mitra, *J. Indian Chem. Soc.*, 1932, **9**, 633). The reaction is characteristic to β-thioketonic esters and this has now been confirmed. It is also important to note that the reaction could not be performed in a perfectly anhydrous condition, the presence of moisture was essential. This suggests that aldehydes at first form additive compounds hydroxysulphides (I) with β-thioketonic esters in thiol phase, which owing to their unstability (*vide* Mitra, *J. Indian Chem. Soc.*, 1933, **10**, 76) give rise to hydroxymercaptans (II) in the presence of mineral acids. These hydroxymeracaptans then lose water intramolecularly to form stable trithio derivatives (III).



(I) (II)

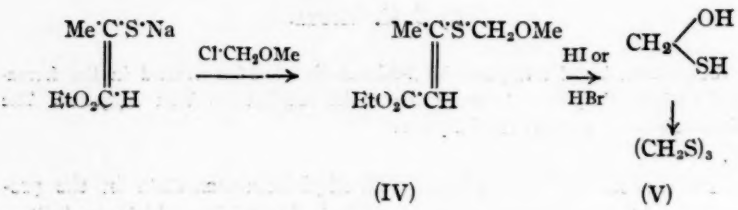


(III)

(R = H, alkyl or aryl).

A similar mechanism can be suggested for the formation of β-trithio derivatives from the interaction of hydrogen sulphide with aldehydes (Fromm and Baumann, *Ber.*, 1889, **22**, 2600 ; 1901, **24**, 1431, 1457 ; 1900, **23**, 60).

Similarly, trithioformaldehyde (V) was obtained by the hydrolysis of the compound (IV), furnished by the condensation of chloromethylether with sodioethyl thioacetoacetate :



(IV) (V)

The results of the reaction of β-thioketonic esters with aldehydes have been given in Table I.

TABLE I.

Esters.	Ph·CHO.	H·CHO.	$C_6H_4(OMe)CHO$. (1 : 4)	$C_6H_3(OH)(OMe)\cdot CHO$. (1 : 3 : 4)
Me·CS·CH(Me)·CO_2Et	$(Ph\cdot CHS)_3$ m. p. 226°	$(CH_2S)_3$ m. p. 218°	$[C_6H_4(OMe)CHS]_3$ m. p. 186°	$(C_6H_3OH\cdot OMeCHS)_3$ m. p. 220° (decomp.)
Me·CS·CH·CO_2Et | $Me_2CH\cdot CH_2$	Do	Do	—	Do
SC : $(CH_2\cdot CO_2Et)_2$	Do	—	—	Do
Me·CS·CH·CO_2Et | CO_2Et	—	—	Do	—

EXPERIMENTAL

Condensation of Aldehydes with β-Thioketonic Esters (General Method).

The β-thioketonic ester was dissolved in ethyl alcohol saturated with hydrogen chloride at 0°. The aldehyde together with a few drops

of water were next added to the mixture which was for a short time kept at 100° and left overnight. The products were identified with those obtained by the interaction of hydrogen sulphide with the corresponding aldehydes, dissolved in alcohol saturated with hydrogen chloride.

β- Trithiobenzaldehyde.—Ethyl methylthioacetoacetate (5 g.) was dissolved in alcohol (20 c.c.) containing benzaldehyde (4 g.). The product was isolated as needles from acetic acid, m.p. 226°. It was also isolated in a similar manner from a mixture of ethyl *iso*butylthioacetoacetate (3 g.) and benzaldehyde (1 g.).

β-Trithioanisaldehyde.—Ethyl methylthioacetoacetate (5 g.) in alcohol (10 c. c.) was mixed with anisaldehyde (3 g.). The compound was isolated as needles from acetic acid, m. p. 186°. [Found : C, 63·0 ; H, 5·6. $(C_3H_8OS)_3$ requires C, 63·1 ; H, 5·3 per cent].

The same compound was isolated from a mixture of diethylthioacetone-dicarboxylate (3 g.) and anisaldehyde (2 g.) as also from a mixture of diethyl thioacetylmalonate (2 g.) and anisaldehyde (2 g.).

β-Trithiovanillin.—Ethyl methylthioacetoacetate (4 g.) was mixed with a solution of vanillin (4 g.) in alcohol (10 c.c.). The compound was isolated as needles from pyridine, m.p. 220°. [Found : C, 56·8 ; H, 4·6. $(C_8H_8O_2S)_3$ requires C, 57·1 ; H, 4·7 per cent].

The same compound was isolated from a mixture of ethyl *iso*butylthioacetoacetate (2 g.) and vanillin (2 g.) as also from a mixture of diethyl thioacetone-dicarboxylate (5 g.) and vanillin (3 g.).

Trithioformaldehyde.—Aqueous formaldehyde (10 c.c. of 40% solution) was added to a solution of ethyl methylthioacetoacetate (10 g.) in ethyl alcohol (40 c.c.) saturated with hydrogen chloride. The whole mass was kept at the room temperature for 4 hours. The precipitate furnished colourless needles from acetic acid, m.p. 218°. [Found : S, 68·8. $(CH_2S)_3$ requires S, 69·5 per cent].

The same compound was isolated from a mixture of ethyl *iso*butylthioacetoacetate (2 g.) and aqueous formaldehyde (5 c.c.).

Ethyl β-Methylmethoxymercapto-α-methylcrotonate.—Ethyl thioacetoacetate (33 g.) was added slowly under ice cooling to emulsified sodium (5 g.) in benzene (100 c.c.) suspension. The mixture was kept at room temperature (15°) for 3 hours and chloromethylether (10 g.) was slowly added. The mixture was boiled under reflux (6 hours). From the benzene extract the fraction, b.p. 120°/12 mm. was collected. (Found : C, 50·3 ; H, 7·5 ; S, 16·6. $C_8H_{14}O_3S$ requires C, 50·5 ; H, 7·3 ; S, 16·8 per cent).

Trithioformaldehyde from Ethyl β-Methylmethoxymercapto-α-methylcrotonate.—Ethyl β-methylmethoxymercapto-α-methylcrotonate (5 g.) was mixed with aqueous hydrobromic acid (*d* 1·87, 30 c.c.) at room temperature (15°). After 2 hours the mixture was kept at 50° (4 hours). The product was then diluted to twice its volume with water and the separated material was washed with alcohol. The residue furnished trithioformaldehyde from pyridine, m.p. 218°.

My sincere thanks are due to Prof. Samuel Smiles, F.R.S. for his keen interest in this investigation and also for the facilities granted.

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ORGANIC ACID—Fe COMPLEX AS A DISTURBING FACTOR IN THE TITRIMETRIC ESTIMATION OF ASCORBIC ACID.

By K. P. Basu and M. C. Nath.

Organic acid—Fe" complexes can act as disturbing factors in the estimation of ascorbic acid with Tillmans' reagent (2 : 6-dichlorophenol-indophenol) even in the acid medium. Quantitative investigation of reducing properties of some of these acid—Fe" complexes has been made. The method of precipitation with mercuric acetate cannot remove citric acid—Fe" completely (elimination = 87%).

Within a year after the introduction of 2 : 6-dichlorophenolindophenol for estimating ascorbic acid in tissue and vegetable extracts by Tillmans, Hirsch and Hirsch (*Z. Lebens*, 1932, **63**, 1) it was pointed out that the presence of sulphydryl compounds, such as cysteine and glutathione interfered with the estimation in the neutral medium at which the titration was usually made. Birch, Harris and Ray (*Biochem. J.*, 1933, **27**, 590; Wolff, Van Eekelen and Emmerie (*Acta Brev. neerl.* Physiol., 1933, **3**, 44) conducted the estimation in the acid medium and did away with the disturbing effect of glutathione and ferrous salts which reduce the indicator in neutral medium. But cysteine, ergothione and tannins still exerted their influence in reducing the reagent. Emmerie (*Biochem. J.*, 1934, **28**, 268) and Emmerie and Van Eekelen (*Biochem. J.*, 1934, **28**, 1153) developed the method of eliminating these disturbing factors present in the trichloroacetic acid extract of the tissues, by precipitation with mercuric acetate. Proteins, which were another source of error in the titration method, were already removed while extracting the tissues with trichloroacetic acid. It was also recorded in the same year (Van Eckelen, *loc. cit.*) that besides these, other reducing substances such as thiosulphates in urine caused marked deviation from the correct result if they were not eliminated (*cf.* Heinemann, *Biochem. J.*, 1936, **30**, 2299). Van Eekelen has shown that mercuric acetate is quite efficient in precipitating thiosulphates and other disturbing factors.

The effect of ferrous salts which were also found to interfere with the estimation of ascorbic acid by Tillmans' reagent was supposed to be eliminated if the titration was carried out in acid medium (*cf.* Birch, *loc. cit.*, Wolff *et al*, *loc. cit.*) and Svirbely and Szent-Györgyi (*Biochem. J.*, 1933, **27**, 279), but results obtained by us are very striking. It has been found that though the ferrous salts of inorganic acids (*e.g.* ferrous sulphate)

have little or practically no influence on the reduction of the indophenol dye in acid medium, ferrous sulphate in presence of organic acids, both dibasic and hydroxy acids, or in other words organic acid—Fe″ complex possesses a strong reducing action on the indicator, even in acid medium.

The present communication deals with the quantitative estimation of the reducing action of some of the organic acid—Fe″ complexes on the Tillmans' reagent. The degree of elimination of this class of reducing complexes by Emmerie's reagent will also be discussed.

EXPERIMENTAL.

The indicator solution was made and standardised according to Harris and Ray (*Biochem. J.*, 1933, **27**, 303). The indicator solution (5 c.c.) was found equivalent to 0·4134 mg. of ascorbic acid. 0·5 C.c. of acetic acid was added to the dye before titration as recommended by Ghosh and Guha (*J. Indian Chem. Soc.*, 1935, **12**, 30).

Experiments were carried out with the dicarboxylic acids *viz.*, oxalic, malonic and succinic and the hydroxy acids *viz.*, malic, tartaric and citric, which are generally present in large quantities in fruits and vegetables.

It will be seen from Table I that as the number of carbon atoms in the dibasic acids increases the reducing capacity of the acid—Fe″ complex diminishes. Succinic acid—Fe″ has practically no reducing property at all. Oxalic acid at the concentration of $M/5$ forms insoluble ferrous salt with $M/5$ ferrous sulphate; hence a concentration of $M/50$ was selected for this acid. It will also be seen that of the three hydroxy acid—Fe″ complexes, citric acid—Fe″ has the greatest reducing property and malic acid—Fe″ the least and it thus appears that in the hydroxy acids the reducing power is directly proportional to the molecular weight of the acids. Blank experiments were always carried out.

It is to be noted that neither the acids nor ferrous sulphate in aqueous solution taken separately, exert any appreciable reducing action on the dye. The reducing action of the acid—Fe″ complexes is shown in Table I. The strength of the ferrous sulphate solution, which was always the same as that of the acids, was always $M/5$ except in the case of oxalic acid where a solution of $M/50$ was used.

It will appear from Table I that when the organic acid and the ferrous salt are taken in equivalent amounts the mixture possesses the greatest reducing properties. Organic acids as well as iron are widely distributed in the vegetable kingdom. It is possible that the reducing action of plant and fruit juices on 2:6-dichorophenol-indophenol may, to

some extent at least, be due to the presence of organic acid—Fe″ complexes as mentioned above. It is absolutely neceessary to eliminate all these disturbing factors before ascorbic acid can be accurately determiued by Tillmans' method.

TABLE I.

(1 C.c. indicator ≡ 0·0827 mg. of ascorbic acid).

Proportion of the vol. of organic acid : ferrous sulphate in the solution.	Vol. of acid—Fe″ complex ≡ 5 c.c. indicator.				
	Oxalic.	Malonic	Malic.	Tartaric.	Citric.
9 :1	4·4 c.c.	3·4 c.c.	6·2 c.c.	4·5 c.c.	2·0 c.c.
8 :2	3·4	2·6	5·4	3·6	1·5
7 :3	2·6	2·1	4·8	3·0	1·2
6 :4	2·4	2·0	4·2	2·6	1·1
5 :5	2·3	1·8	3·8	2·4	1·0
4 :6	2·4	2·1	4·8	2·8	1·1
3 :7	4·1	2·6	5·9	3·4	1·2
2 :8	6·6	3·3	6·8	4·1	1.5
1 :9	12·25	4·6	7·8	4·7	1·9

Experiments were then carried out with a view to remove these acid—Fe″ reducing complexes by precipitation with mercuric acetate. Results are shown in the following table.

TABLE II.

(1. C.c. indicator ≡ 0·0827 mg. of ascorbic acid).

100 C.c. solution containing.	Vol. ≡ 2 c.c. of indicator.	Total vol. of indicator ≡ 100 c.c. solution.	Equiv. weight of ascorbic acid.
M/5-citric acid (5 c. c.) and 5 c.c. ferrous sulphate (*M*/5)	2·6 c.c.	77·0 c.c.	6·37 mg.
M/5 citric acid (5 c.c.) and 5 c.c. ferrous sulphate treated with mercuric acetate and hydrogen sulphide	20·0	10·0	0·83

It appears from Table II that only 87% of the reducing complex are eliminated by treatment with mercuric acetate.

Thus it is evident that due to the presence of various reducing factors, the titrimetric method of Tillmans for estimating ascorbic acid cannot give very accurate results whatever precautions are taken for eliminating them. A recent observation by Miller and Robbins (*Biochem. J.*, 1937, **31**, 1) also shows that results with mercuric acetate are unsatisfactory.

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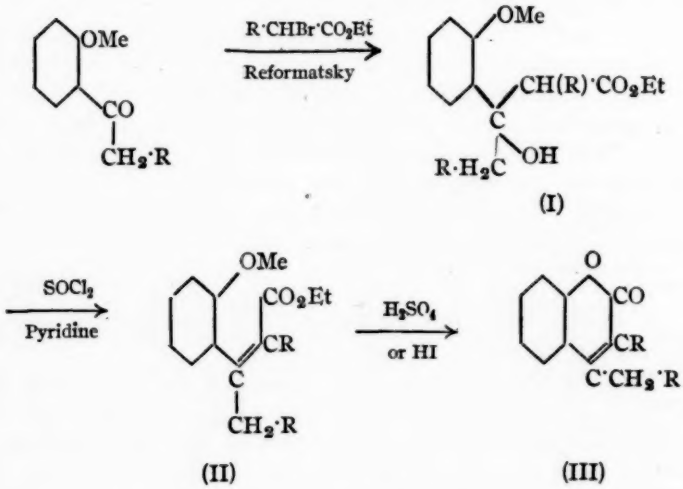
SYNTHESIS OF COUMARINS FROM *o*-HYDROXY—ARYLALKYL KETONES. PART I.

By Duhkhaharan Chakravarti and Brojeswar Majumdar.

The condensation product of *o*-methoxy-arylalkyl ketones with α-halogenated fatty esters is dehydrated yielding the unsaturated ester which easily forms coumarin derivatives either with sulphuric acid or hydriodic acid. The syntheses of 3-methyl-4-ethyl-6-chlorocoumarin and 3 : 7-dimethyl-4-ethyl-6-chlorocoumarin by the condensation of ethyl α-bromopropionate with 2-methoxy-5-chlorophenylethyl ketone and 2-methoxy-5-chloro-4-methylphenylethyl ketone respectively have been described.

In course of the synthetical studies on coumarins and chromones, 4-ethylcoumarins were formed and it was thought advisable to synthesise authentic 4-ethylcoumarins by unambiguous methods. *o*-Hydroxy-arylalkyl ketones, which are readily available either by Hoesch's reaction or by Fries' rearrangement of acylated phenols, may readily be converted to coumarin derivatives in the following way :—



(where R = H or an alkyl group).

The hydroxy-ester (I) is contaminated with the unsaturated ester (II) and hence the hydroxy-ester is always dehydrated with thionyl

chloride in the presence of pyridine. The ring-closure of (II) to (III) may be effected by heating with hydriodic acid (*d* 1·7) or by keeping overnight with cold concentrated sulphuric acid (*d* 1·84). In both cases the yield of the coumarin is almost quantitative.

The *o*-hydroxy-arylalkyl ketones have been used by Kostanecki and later on by Robinson for the synthesis of chromone derivatives. Heilbron and co-workers have found that coumarins are also formed by the action of the *o*-hydroxy-arylalkyl ketones with acid anhydrides in the presence of the sodium salt of the acid (*J. Chem. Soc.*, 1933, 1263; 1934, 1311, 1581; 1936, 296; *cf.* Chakravarti and Bagchi, *J. Indian Chem. Soc.*, 1936, **13**, 689). Stoermer and Friderici (*Ber.*, 1908, **41**, 324) and Mitter and Paul (*J. Indian Chem Soc.*, 1931, **8**, 271) submitted *o*-methoxybenzophenones to Reformatsky's reaction but the yields of the coumarins obtained were extremely poor.

This paper describes the synthesis of 3-methyl-4-ethyl-6-chlorocoumarin and 3 : 7-dimethyl-4-ethyl-6-chlorocoumarin from ethyl α-bromopropionate and the methoxy derivatives of the *o*-hydroxypropiophenones, obtained by Fries' rearrangement of the propionates of 4-chlorophenol and 4-chloro-3-methylphenol.

This method bids fair to be a general method for the synthesis of 3:4-dialkyl coumarin derivatives, which cannot be synthesised by methods known at present. The yields of the intermediate products and of the coumarins are highly satisfactory. Further work is in progress.

Experimental.

2-Methoxy-5-chlorophenylethyl ketone was obtained from 2-propionyl-4-chlorophenol (Wittig, *Annalen*, 1925, **446**, 186) with methyl iodide in alcoholic sodium ethoxide. It distils at 135-40°/6 mm. as a thick colourless oil, which solidifies on long standing, m.p. 41-42°. (Found : Cl, 17·64. $C_{10}H_{11}O_2Cl$ requires Cl, 17·88 per cent).

Ethyl α-Methyl-β-ethyl-β-hydroxy-β-(2-methoxy-5-chloro)-phenylpropionate.—A mixture of 2-methoxy-5-chlorophenylethyl ketone (7 g.), freshly distilled ethyl α-bromopropionate (7 g.), zinc wool (3 g.) and benzene (20 c.c.) was heated on a water-bath for 3 hours, when the zinc dissolved. The solution was then poured into ice-cold dilute sulphuric acid, the benzene layer separated, washed with dilute sodium carbonate solution and then with water and dried. On removing benzene it was collected at 155-60°/7 mm. as a thick yellow oil, yield 6·5 g. On standing the oil partly solidified and the solid was collected and crystallised from rectified spirit as colourless needles, m.p. 71°. (Found : Cl, 11·79. $C_{15}H_{21}O_4Cl$ requires Cl, 11·8 per cent).

Ethyl 2-Methoxy-5-chloro-α-melhyl-β-ethylcinnamate.—The product of condensation obtained above (5 g.) was converted into the unsaturated ester with thionyl chloride (2 g.) in the presence of pyridine (3 g.) in dry ethereal solution (100 c.c.). The ethereal layer after decomposing excess of thionyl chloride with ice was washed with dilute hydrochloric acid, with alkali and then with water. On removing ether the product was distilled at 163°/6 mm. as a colourless oil which did not solidify on standing, yield quantitative.

3-Methyl-4-ethyl-6-chlorocoumarin was obtained from the above unsaturated ester (1 g.) by heating with hydriodic acid (*d* 1·7, 5 c.c.) in an oil-bath at 140-50° for 2 hours. The oil obtained on pouring the mixture into water was extracted with ether, the ethereal extract washed repeatedly with dilute soda solution and finally with water. On removing ether the coumarin was obtained as colourless needles, which were recrystallised from rectified spirit, m.p. 94°, yield 0·8g. (Found : Cl, 15·77. $C_{12}H_{11}O_2Cl$ requires Cl, 15·95 per cent). It dissolves in warm alkali and is obtained unchanged on acidification. The ring-closure may also be effected with good yield by keeping overnight the unsaturated ester with a few c.c. of sulphuric acid (*d* 1·84).

Formation of 3:7-Dimethyl-4-ethyl-6-chlorocoumarin from 2-Methoxy-4-methyl-5-chlorophenylethyl ketone and Ethyl α-bromopropionate.—2-Methoxy-5-chloro-4-methylphenylethyl ketone, prepared from 2-propionyl-4-chloro-5-methylphenol (Rosenmund and Schnurr, *Annalen*, 1928, **460**, 84) with methyl iodide in alcoholic sodium ethoxide was crystallised from alcohol as colourless plates, m.p. 74°. (Found : Cl, 16·9. $C_{11}H_{13}O_2Cl$ requires Cl, 16·7 per cent).

The ketone (8 g.) and ethyl α-bromopropionate (8 g.) were condensed as above using zinc wool (4 g.) in dry benzene solution and the product (8 g. b.p. 170°/5 mm.) was converted into the unsaturated ester (b.p. 165°/5 mm.) with thionyl chloride in the presence of pyridine, yield quantitative. The unsaturated ester (2 g.) was heated in an oil-bath at 140-45° for 2 hours with hydriodic acid (*d* 1·7, 6 c.c.). The solid separating on pouring the solution into water was crystallised from rectified spirit as colourless needles, m.p. 121°, yield 1·5 g. It dissolves in warm alkali to a yellow solution and is recovered on acidification. (Found : Cl, 14·78. $C_{13}H_{13}O_2Cl$ requires Cl, 15·01 per cent).

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NATURAL FLAVONES. PART I. THE CONSTITUTION OF GARDENIN.

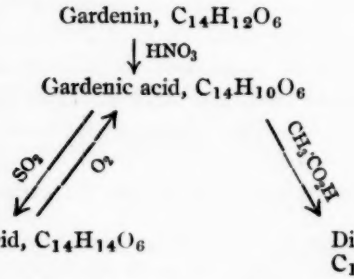
By Prafulla Kumar Bose and Rajendralal Nath.

Gardenin was regarded by Stenhouse and Groves to be $C_{14}H_{12}O_6$. Experimental evidence, which has been brought forward in this paper, shows that gardenin has the molecular formula $C_{21}H_{22}O_9$ and is either 5-hydroxy-3 :6 :8 :3′ :4′ :5′-hexamethoxyflavone or its isomer 5-hydroxy-3 :7 :8 :3′ :4′ :5′-hexamethoxyflavone. This is the first instance of a derivative of heptahydroxyflavone found in nature.

Gardenin is the yellow colouring matter present in Dikamali gum, which is a resinous exudation of the leaf-bud of *Gardenia lucida*, Roxb. (= *G. resinifera* Roth or *G. gummifera* Linn.) belonging to the N. O. Rubiaceæ.

Sanskrit writers recommend the gum in fever, dyspepsia, flatulence and chronic skin diseases, while it is much prescribed by Hakims in Bombay in dyspepsia attended with flatulence (Dymock, Warden and Hooper, *Pharm. Ind.*, II. p. 207 ; Dymock, *Pharm. J.*, 1876, **7**, 491).

More than eighty years ago Stenhouse reported to the Royal Society of London the presence of a golden yellow crystalline substance in Dikamali gum, which was named gardenin. Flückiger (*Pharm. J.*, 1877, **7**, 589) isolated gardenin from the same source and his analytical data agreed with the formula $C_{23}H_{30}O_{10}$. Stenhouse and Groves (*J. Chem. Soc.*, 1877, 552), however, observed that pure gardenin melts at 163-64°, and their analyses agreed with the formula $(C_5H_5O_2)_x$. Further investigation by the same workers (*Annalen*, 1880, **200**, 311) indicated that the analytical data were in better agreement with the formula $C_{14}H_{12}O_6$, although they did not determine the molecular weight of the substance. Oxidation of gardenin with nitric acid gave an orange-red crystalline substance, $C_{14}H_{10}O_6$ (m.p. 223°) which was called gardenic acid. Reduction of this substance led to a yellow product, $C_{14}H_{14}O_6$ (m.p 190°), called hydrogardenic acid, which could be converted by careful oxidation into the parent acid. The latter was suspected to be a quinone and gave a diacetyl derivative, m.p. 230-44°, on being boiled with acetic acid. The main findings of Stenhouse and Groves may be summarised thus :



The authors, however, did not propose any definite constitutional formula for gardenin based on the above results.

We took up the investigation of this substance fifty-six years after the last publication of researches on gardenin. The method recommended by Stenhouse and Groves for the isolation of crude gardenin was followed. The process of purification was, however, modified (*vide* Experimental). Our gardenin was found to melt at 161°-62° (uncorr.) and the melting point could not be raised by crystallisations from different solvents. The elementary analyses of our gardenin gave values which were very close to those found by Stenhouse and Groves, but not identical.

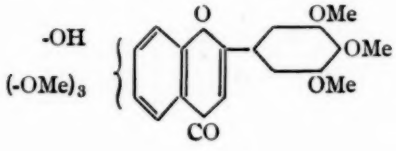
The analytical values obtained by different investigators are as follows:

Flückiger.	Stenhouse and Groves.		Present authors.
	I	II	
C=59·47 (mean)	61·91	60·85	60·16, 60·61 per cent.
H=6·71 („)	5·45	4·75	5·22, 5·3 „

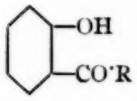
We observed, moreover, that gardenin contains a high percentage of methoxy group, a fact which had escaped the notice of earlier workers. Our analytical data agreed with the formula $(C_7H_7O_3)_x$ for gardenin. A molecular weight determination in benzene by the cryoscopic method did not give reliable results owing to the low solubility of gardenin in this solvent. Stenhouse and Groves had observed that gardenin could be acetylated or benzoylated, but did not isolate the acetyl or benzoyl derivatives. We found that gardenin gives, under the usual condition of acetylation, a monoacetylgardenin, m.p. 136°, which had a mean molecular weight of 454. The parent compound, therefore, must have a molecular weight of about 412. In other words the molecular formula of gardenin must be $C_{21}H_{20}O_9$ or $C_{21}H_{22}O_9$.

Gardenin is insoluble in cold 5% alkali, but dissolves to a yellow

solution on heating. Evidently gardenin possesses weak phenolic properties. The presence of phenloic OH group is further proved by acetylation and reaction with ferric chloride, which imparts an olive-green colour to an alcoholic solution of gardenin. It has no reducing action on alkaline *o*-dinitrobenzene or ammoniacal silver nitrate solution. It is optically inactive. Tests for the methylenedioxy group and quinone were negative. A red colour was produced on treating an alcoholic solution of gardenin with magnesium and hydrochloric acid, suggesting that gardenin might belong to the flavone group. Since gardenin contains one hydroxy and six methoxy groups, such a supposition would imply that gardenin is a derivative of heptahydroxyflavone, a representative of which has not, up till now, been found in nature or synthesised. Assuming that gardenin is a flavone, it should be expected to undergo facile hydrolysis by alkali. As a matter of fact 15-20% alkali decomposed gardenin and two different crystalline substances were isolated from the reaction mixture. One of these was found to be identical with trimethylgallic acid. The partial formula for gardenin can, therefore, be represented by (I). This also leads to the acceptance of $C_{21}H_{22}O_9$ as the correct molecular formula for gardenin.



(I)



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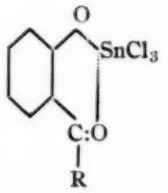
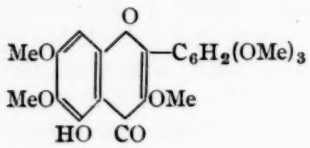


(III)

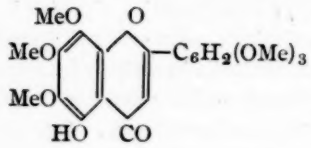
Next we attempted to locate the position of the free hydroxyl group. The stability of an alkaline solution of gardenin to aerial oxidation indicates that the -OH group is not in position 3 of the flavone nucleus. Evidently it must occupy one of the four positions, namely 5, 6, 7 or 8 of the chromone nucleus. That the position 5 might be occupied by the -OH group was suggested by the fact that in partially methylated hydroxyflavones, flavonols and flavanones occurring in nature, the -OH group in position 5 is, as a rule, never found in the methylated condition. It is, moreover, well known that methylation of the hydroxyl group in position 5 proceeds with great difficulty. This was also found to be the case with gardenin. A positive proof of the position of the hydroxyl group was, however, furnished by an examination of the condensation product of gardenin with stannic chloride. The ratio of Sn: Cl in

the complex salt was found to be nearly 1 : 3. Pfeiffer (*Annalen*, 1913, **398**, 137) has shown that a compound of the type (II) reacts with stannic chloride in benzene solution forming (III), whereas if the -OH group be not adjacent to the -CO·R group, then double compounds of the type $HO{\cdot}C_6H_4{\cdot}CO{\cdot}R{\cdot}SnCl_4$ are formed. The formation of the $SnCl_3$-complex definitely establishes the position of the OH group, which must occupy position 5.

The positions of the remaining three methoxy groups now remain to be settled. There are four possibilities, namely (IV), (V), (VI) or (VII). Compounds of the type (IV) and (V) on demethylation would give rise to



(IV)



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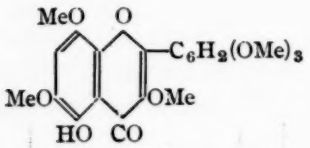

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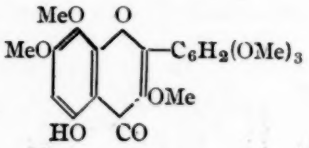
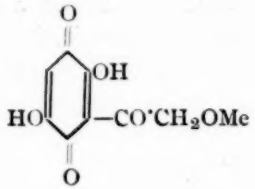


(VII)

heptahydroxyflavones having three contiguous OH groups in positions 5, 6 and 7. Such compounds should be expected to give a positive Bargellini's test. In fact an alcoholic solution of demethylated gardenin gave a brown precipitate with sodium amalgam, but not the green precipitate characteristic of flavones having OH groups in positions 5, 6, and 7 (Bargellini, *Gazzetta*, 1919, **49**, *ii*, 47; Baker, *J. Chem. Soc.*, 1928, 1030; Baker, Nodzu and Robinson, *J. Chem. Soc*, 1929, 83). It seems, therefore, unlikely that gardenin should have the structure (IV) or (V). The choice consequently must lie between (VI) and (VII).

The second product of alkaline hydrolysis of gardenin was also examined with a view to throw more light on the constitution of gardenin. This substance separates as a deep red dipotassium salt from the reaction mixture. Treatment with hydrochoric acid liberated a chocolate coloured compound, m.p. 158-60°, which is fairly soluble in water. It has acidic reactions and gives a dirty violet colour with ferric chloride. On reduction with sulphurous acid it is converted into a yellow compound, m.p. 175-76°. This

is also acidic to litmus and gives a violet ferric chloride reaction. Unlike the parent substance, however, it reduced alkaline *o*-dinitrobenzene in the cold (*cf.* Bose, *J. Indian Chem. Soc., Ray Com. Vol.*, 1933, p. 65) showing the presence of -OH groups in *ortho-* or *para*-positions to one another. On keeping it exposed to the atmosphere it is slowly transformed into the parent substance, m. p. 158-60°. The analytical data of these two substances as also their properties are in agreement with the formulae (VIII) and (IX) or (X) and (XI) or their isomers. We are attempting to synthesise compound (IX).



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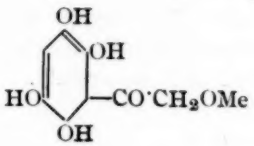

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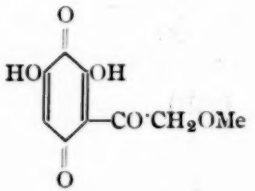
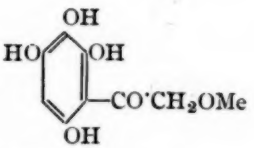


(X)

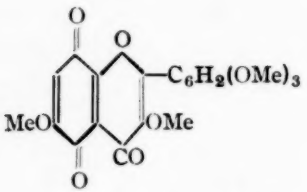


(XI)

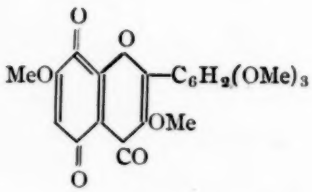
Incidentally we have examined the so-called 'gardenic acid' of Stenhouse and Groves and its reduction product, 'hydrogardenic acid.' 'Gardenic acid' prepared by us by following the method of earlier workers was found to melt at 222-24° (*lit.* 223°) and agreed with the formula $C_{20}H_{18}O_9$. It liberated iodine from acidified potassium iodide solution showing that it is a quinone, We propose the name gardeninone for this compound, which is evidently formed by loss of a methyl group. From the mother-liquor (acetic acid) of gardeninone we obtained a small quantity of another product, m. p. 238-40°, which was not further examined and which is probably the so-called 'diacetyl gardenic acid' of Stenhouse and Groves. Further oxidation of gardeninone by nitric acid (*d* 1·25) led to a colourless compound, m. p. 96-97°, which was identified as 1-nitro -3 : 4 : 5-trimethoxybenzene. This compound is no doubt formed by

the nitration of the side-chain of gardeninone, since trimethylgallic acid also produced the same compound under identical experimental conditions. Prolonged contact with nitric acid gave a compound, m. p. 116-17°, which is believed to be 1 : 2-dinitro-3 : 4 : 5-trimethoxybenzene, since it responded to the colour reactions of *o*-dinitro-compounds (Bose and Sundar Ram, *J. Indian Chem. Soc.*, 1935, **12**, 687).

Reduction of gardeninone by sulphurous acid gave a deep yellow crystalline compound, m. p. 184-85° (the 'hydrogardenic acid' of Stenhouse and Groves). This quinol, called gardeninol by us, gave the tests of a polyhydroxybenzene derivative (Bose, *loc. cit.*) and formed a diacetyl derivative. Assuming the structure (VI) or (VII) for gardenin, the formulæ for gardeninone and gardeninol may be represented by (XII) or (XIII) and (XIV) or (XV) respectively.



(XII)



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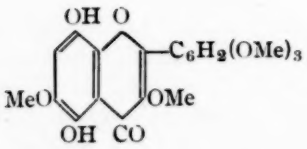
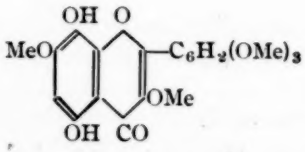


(XIV)



(XV)

EXPERIMENTAL.

Purification of Gardenin.—Crude gardenin, obtained by the method of Stenhouse and Groves (*loc. cit.*), was washed with cold carbon disulphide to remove the greater portion of adhering resins. It was then dissolved in chloroform and diluted with rectified spirit, when yellow needles separated out. This process was repeated three or four times till the m.p. became constant. Finally it was crystallised from absolute alcohol; m.p.

161-62°. (Found : C, 60·16, 60·61 ; H, 5·22, 5·3 ; OMe, 44·24. $C_{21}H_{22}O_9$ requires C, 60·29 ; H, 5·26 ; OMe, 44·49 per cent).

Acetylgardenin.—A mixture of gardenin (0·2 g.), freshly fused sodium acetate (1·5 g.), acetic anhydride (4.c.c.) was gently boiled for 5 hours under reflux. The acetyl derivative was separated in the usual manner and crystallised from benzene-ligroin in very pale yellow needles, m.p. 136°. [Found : C, 59·55 ; H, 5·26 ; acetyl, 8·06 ; M.W. (cryoscopic in benzene), 460·4, 448. $C_{23}H_{24}O_{10}$ requires C, 60·0; H, 5·22 ; acetyl, 9·34 per cent. M.W., 460).

Hydrolysis of Gardenin.—Gardenin (1 g.) was refluxed with alcoholic potassium hydroxide (15%, 20 c.c.) for 7 hours. Gardenin gradually went into solution with an orange colour which afterwards turned red with the progress of the reaction. The mixture was left overnight and the red crystalline precipitate, which had separated out, was filtered off the next day and washed twice with absolute alcohol. This was then dissolved in the minimum quantity of water and the solution was diluted with alcohol when the red potassium salt crystallised out. This process was repeated four times and the compound was then analysed. It could not be completely freed from a trace of potassium carbonate, which was detected in the usual manner. (Found : K, 29·09. $C_9H_6O_6K_2$ requires K, 27·08 per cent).

Isolation of Trimethylgallic Acid.—The filtrate from the potassium salt was acidified with hydrochloric acid and evaporated to dryness on a water-bath. The residue was washed with a little cold water and then crystallised from dilute methyl alcohol, sublimed in high vacuum and the sublimate crystallised from dilute methyl alcohol, when colourless needles were obtained ; m.p. 165-66°. It dissolved in cold sodium bicarbonate solution and did not depress the m.p. of synthetic trimethylgallic acid.

Isolation of the Second Product of Hydrolysis.—The red potassium salt was dissolved in the minimum quantity of water and just acidified with dilute hydrochloric acid (congo-red). The solution was allowed to concentrate in vacuum over sulphuric acid and potassium hydroxide. The dark brown crystals, which separated out, were collected, washed with a little cold water and recrystallised from water at room temperature in a similar manner. It formed dark chocolate crystals, m.p. 158-60°. The substance was acidic to litmus and gave a dirty violet colour with ferric chloride. It was indifferent towards alkaline *o*-dinitrobenzene. (Found: C, 50·50, 50·56; H, 3·9, 3·55; OMe, 14·65. $C_9H_8O_6$ requires C, 50·94; H, 3·77; OMe, 14·62 per cent).

Reduction of the above Compound : Formation of Tetrahydroxy-ω-methoxyacetophenone.—0·2 G. of the above substance was dissolved in rectified spirit and the solution saturated with sulphur dioxide. The mixture was left overnight in a cool place and then concentrated on the water-bath, when a brown-yellow crystalline compound separated out. This was washed with a little cold water and dried (0·13 g.). It was sublimed in high vacuum (150°-55°/0·05 mm.) and then crystallised from alcohol in yellow needles, m.p. 175-76°. It gave a violet ferric chloride reaction and a positive test with alkaline *o*-dinitrobenzene. (Found : C, 50·80; H, 4·98. OMe, 14·55. $C_9H_{10}O_6$ requires C, 50·47; H, 4·67; OMe, 14·49 per cent).

Gardeninone.—Finely powderd gardenine (1 g.) was placed in a basin previously cooled in ice-water. To this was added nitric acid (*d* 1·25, 10 g.) The mixture was thoroughly stirred. The yellow colour of gardenin changed to orange in 2-3 minutes and finally became deep orange-red. The reaction was continued for about 10 minutes and the completion of the reaction was ascertained by observing the crystals under the microscope, the absence of the yellow crystals of gardenin indicating the end. The temperature of the reaction should not exceed 25°, otherwise the yield is diminished owing to further oxidation of gardeninone. Temperatures below 10° again make the reaction very sluggish. The crude gardeninone was thoroughly washed with water till free from nitric acid and dried (0·5 g.). This was then crystallised from glacial acetic acid as orange crystals, m.p. 222-24°. (Found : C, 60·0 ; H, 4·72 ; OMe, 38·34. $C_{20}H_{18}O_9$ requires C, 59·70; H, 4·48; OMe, 38·56 per cent).

Oxidation of Gardeninone.—Gardeninone was boiled with nitric acid (*d* 1·25) till the former went into solution and the solution became colourless. The clear solution was then rapidly cooled and kept in an ice chamber for several hours. The colourless crystals which separated out were collected and crystallised from dilute methyl alcohol. It was further purified by sublimation at 90°-100°/0·05 mm.; m.p. 97°.

Nitration of Trimethylgallic Acid.—Trimethylgallic acid was boiled with nitric acid (*d* 1·25) till effervescence ceased and the acid went into solution. On cooling a crystalline substance was obtained, which was purified as described above. It melts at 97° and did not depress the m.p. of the compound described above. The m.p. of 1-nitro-3 : 4 : 5-trimethoxybenzene given in literature is 99-100°.

Formation of 1 : 2-Dinitro-3 : 4 : 5-trimethoxybenzene.—If gardenin be boiled with an excess of nitric acid of the same strength for a longer period

and the clear colourless solution is allowed to stand for several days at room temperature, a crystalline substance is obtained. On crystallisation from dilute alcohol, it forms almost colourless crystals, m. p. 116-17°. It gives a violet colour with uric acid and alkali, showing that it is an *ortho*-dinitro compound, which cannot be anything else but the above compound.

Gardeninol.—Through a suspension of gardeninone in rectified spirit, a current of sulphur dioxide was passed till the quinone went into solution. On concentration and cooling yellow crystals were obtained. Recrystallised from alcohol it formed yellow needles, m. p. 184-85° (the m. p. of 'hydrogardenic acid' of Stehhouse and Groves is 190°). It is not acidic to litmus but dissolves in dilute alkali with a brown colour. The ferric chloride reaction is deep olive-green. *o*-Dinitrobenzene gives a dirty violet colour in presence of cold dilute alkali. (Found: C, 59·29; H, 5·21. $C_{20}H_{20}O_9$ requires C, 59·4 ; H, 4·95 per cent).

Diacetylgardeninol.—The compound was prepared in the usual manner and formed colourless crystals (alcohol), m.p. 146-47°. (Found: C, 59·03; H, 4·65; OMe, 31·42. $C_{24}H_{24}O_{11}$ requires C, 59·02, H, 4·92 ; OMe, 31·77 per cent).

Methylgardenin.—Gardenin (1 g.) was dissolved in methyl alcohol (50 c.c) and sodium hydroxide (25%, 8 c.c.) was added. The mixture was mechanically stirred and 18 g. of dimethylsulphate and 24 c.c. of sodium hydroxide (25%) were added drop by drop from dropping funnels, so that the solution remained faintly alkaline. After the completion of the reaction, the mixture was diluted with a large volume of water and the precipitate filtered off. The crude methylgardenin still contained considerable quantity of the parent substance and was again methylated in a similar manner. This process was repeated three times. The pale yellow product was finally crystallised from dilute methyl alcohol, m. p. 102° (after drying for 3 hours at 50°-60° in vacuum). (Found: C, 60·60* ; H, 5·34. $C_{22}H_{24}O_9$ requires C, 61·11; H, 5·55 per cent).

Demethylation of Gardenin.—A mixture of gardenin (0·5 g.), hydriodic acid (*d* 1·7, 8 c.c.) and phenol (1 c. c.) was gently boiled for 1·5 hours. The product was poured into very dilute sodium bisulphite solution and

* Highly methylaled flavones usually give low values (*cf.* Heap and Robinson, *J. Chem. Soc.*, 1929, 73; Baker, Nodzu, and Robinson, *ibid.*, 1929, 81; Freudenberg, Fickentocher and Wenner, *Annalen*, 1925, **442**, 314).

the yellow precipitate collected and washed with water. Although it is easily soluble in hot alcohol and acetone, attempts to obtain it in the crystalline form have not been successful. It decomposed above 300° and gave a brown precipitate with sodium amalgam in alcoholic solution.

Condensation of Gardenin with Stannic Chloride.—Gardenin (0·5 g) was dissolved in dry benzene and stannic chloride (1 c.c.) quickly added. The mixture was refluxed for 4 hours. The red precipitate was quickly filtered off, washed with dry benzene and dried over sulphuric acid, paraffin and potash in a desiccator. It does not melt below 335°. (Found: Sn : Cl = 1 : 2·9. Calc. Sn:Cl = 1:3).

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MERCURATION OF ACETYLURETHANE AND ITS SUBSTITUTED AMIDES.

By L. D. Shah.

The preparation and properties of the mercury derivatives of acetylurethane and its substituted amides have been described by following the method of Naik and Shah.

In view of the molecular structure of acetylurethane Me·CONH·COOEt, the hydrogen attached to the nitrogen atom may be expected to be replaceable by metals. The present paper deals with the preparation and properties of the mercury derivatives of acetylurethane and its substituted amides obtained by the action of mercury acetamide in aqueous or alcoholic solution. The following substances have been investigated.

(1) Acetylurethane, (2) acetylphenylcarbamide, (3) acetyl-*m*-tolylcarbamide, (4) acetyl-*o*-tolylcarbamide, (5) acetyl-*p*-tolylcarbamide, (6) acetyl-α-naphtylcarbamide, (7) acetyl β-naphthylcarbamide, (8) acetyl 1: 3: 4- xylyl carbamide, (9) acetyl-*p*-anisylcarbamide, (10) phenylurethane. All give compounds of the general formula $(Me{\cdot}CONCOR)_2Hg$ where R may be a phenyl, *o*-, *m*-, *p*-tolyl, naphthyl or xylyl group. In the case of compound (10) mercury *bis*-phenylurethane was obtained. The method used for the mercuration of these amides is the one used for the first time by Naik and Shah (*J. Indian Chem. Soc.*, 1931, **8**, 29).

The compounds are decomposed by dilute hydrochloric acid and by hydrogen, ammonium or sodium sulphides, the corresponding mercury salt being formed. With potassium iodide the following reaction occurs:—

$$(Me{\cdot}CONCOR)_2Hg + 4KI + 2H_2O \longrightarrow 2MeCONHCOR + 2KOH + K_2HgI_4.$$

Phenylhydrazine and hydrazine hydrate liberate metallic mercury.

Experimental.

Mercury bis-*acetylurethane* was precipitated when an aqueous solution of mercury acetamide and acetylurethane were mixed. It crystallises from hot water in colourless needles, m. p. 174°. (Found: N, 6·46; Hg, 43·72. $C_{10}H_{16}O_6N_2Hg$ requires N, 6·09; Hg, 43·48 per cent).

Potassium iodide liberates 1·87 equivalents of alkali as against 2 equivalents required. Ether extracts acetylurethane from the solution obtained

with 0·25 *N*-HCl. With phenylhydrazine mercury is at once liberated with evolution of nitrogen.

Mercury bis-*acetylphenylcarbamide* was precipitated when mercury acetamide and acetylphenylcarbrmide were heated under reflux in methyl alcohol, m.p. 205-206° (decomp.). (Found: N, 10·01; Hg, 35·88. $C_{18}H_{18}O_4N_4Hg$ requires N, 10·11; Hg, 36·1 per cent).

Acetyl-m-*tolylcarbamide* was formed by heating acetylurethane and *m*-toluidine in equimolecular proportions for 30 minutes at 160°. The solid separating on addition of water was dissolved in hot acetic acid and cooled. Di-*m*-tolylurea, which separated on cooling, was removed and the filtrate diluted with water to precipitate the required amide which was recrystallised from methyl alcohol, m. p. 128°. (Found: N, 14·37. $C_{10}H_{12}O_2N_2$ requires N, 14·58 per cent).

With mercury acetamide in methyl alcoholic solution, it forms *mercury acetyl -m- tolylcarbamide*, m.p. 196-97°. (Found: N, 10·05; Hg, 34·79. $C_{20}H_{22}O_4N_4Hg$ requires N, 9·65 ; Hg, 34·36 per cent).

Mercury bis-*acetyl*-o-*tolylcarbamide* was similarly prepared, m.p. 209-10°. (Found: N, 9·58; Hg, 34·04. $C_{20}H_{22}O_4N_4$ Hg requires N, 9·65; Hg, 34·36 per cent). Treatment with potassium iodide and hydrochloric acid reforms the amide.

Mercury bis-*acetyl* p-*tolylcarbamide* has m.p. 227-28° (with rapid decomposition). (Found : N, 9·78 ; Hg, 34·22. $C_{20}H_{22}O_4N_4Hg$ requires N, 9·65 ; Hg, 34·36 per cent).

Mercury bis-*acetyl*-α-*napthylcarbamide* has m. p. 225-28° (decomp.). (Found: N, 8·31; Hg, 30·11. $C_{26}H_{22}O_4N_4Hg$ requires N, 8·56; Hg, 30·58 per cent).

Mercury bis-*acetyl*-β-*naphthylcarbamide* has m.p. 215-16°. (decomp). (Found: N, 8·79; Hg, 29·44. $C_{26}H_{22}O_4N_4Hg$ requires N, 8·56; Hg, 30·58 per cent).

Acetyl-1:3:4-*xylylcarbamide* was extracted by ethyl acetate from the product formed by heating equimolecular proportions of acetylurethane and 1:3:4-xylidine at 160° per 1 hour and pouring into water. This is identical with the compound prepared from 1:3:4-xylylcarbamide and acetyl chloride in pyridine solution.

Mercury bis-*acetyl*-1:3:4-*xylylcarbamide* has m.p. 231-32° (decomp.). (Found: N, 9·15 ; Hg, 33·12. $C_{22}H_{26}O_4N_4Hg$ requires N, 9·18; Hg, 32·79 per cent).

Acetyl p-*anisylcarbamide* was formed by heating acetylurethane and *p*-anisidine in equimolecular proportions at 160° for 2 hours. It separates dilute acetic acid in needles, m. p. 172-73°. (Found: N, 13·38. $C_{10}H_{12}O_3N_2$ requires N, 13·46 per cent).

Mercury bis-*acetyl*-p-*anisylcarbamide* has m. p. 222° (decomp.). (Found: N, 8·87; Hg, 32·93. $C_{20}H_{22}O_6N_4Hg$ requires N, 9·12; Hg, 32·57 per cent).

Mercury bis-*phenylurethane* was precipitated by adding water to the solution obtained by heating mercury acetamide and phenylurethane in methyl alcohol for 30 minutes, m. p. 203°. (Found: N, 5·45; Hg, 37·47. $C_{18}H_{20}O_4N_2Hg$ requires N, 5·30; Hg; 37·87 per cent).

In conclusion the author wishes to express his gratitude to Dr. T.S. Wheeler for his kind interest throughout the course of this work. He also wishes to thank the Bombay University for a grant in aid of this investigation.

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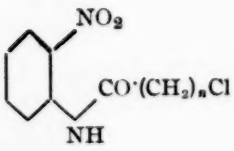
SYNTHESIS OF QUINAZOLINES (AND BENZOGLYOXALINES). PART V.

By Aftab Ahmed, Kartar S. Narang and Jnanendra Nath Ray.

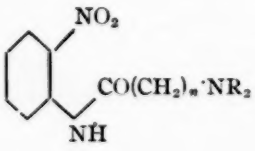
Certain quinazolines and benzoiminazoles have been synthesised with an alkylamino side-chain. In the benzoiminazole series a basic tertiary nitrogen situated at the β-carbon atom in the chain loses the nitrogen complex in the form of the corresponding secondary base and the conjugated unsaturated product, thus produced, undergoes polymerisation. 3 :4-Methylenedioxy-6-aminoacetylbenzylamine is remarkably stable and does not undergo ring-closure.

A description is included of a substance which can be called as methylenedioxy-*iso*vasicone.

In the present investigation, an attempt has been made to synthesise quinazolines related to vasicine and glyoxalines with an aminoalkyl side-chain so that the physiological action of these compounds can be fully investigated. β-Substituted phenylethylamines have well marked sympathomimetic action. Chatterji (*J. Chem. Soc.*, 1929, 2965) has prepared β-benziminazoylethylamine which, however, failed to show any pressor action even in relatively large dose. For the purpose of the synthesis of the benziminazoles, *o*-nitroaniline has been condensed with chloroacetyl and β-chloropropionyl chloride to give (I). The substances of the type (I) were then condensed with piperidine or diethylamine to give (II)$_2$ The amine related to (II) cyclised



(I)



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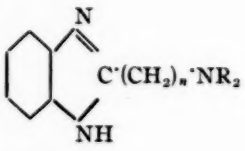
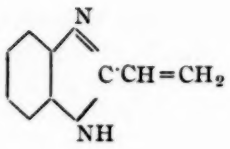
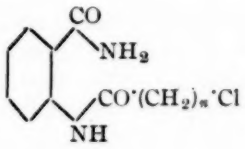


(III)

to the corresponding glyoxaline (III) on being heated with sodium acetate and acetic acid. In the case where $n=2$, the amine related to (II) was smoothly produced but under all conditions of cyclisation the aminoalkyl side-chain or piperidine residue was extruded resulting in the production of a polymer of the substance (IV)

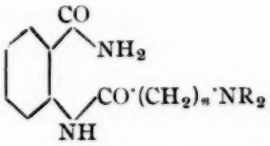


(IV)

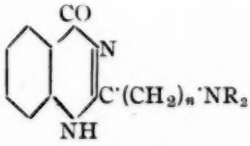


(V)

For the synthesis of the quinazolines, *o*-aminobenzamide was condensed with chloroacetyl and β-chloropropionyl chloride and the products (V) were condensed with diethylamine or piperidine to (VI).

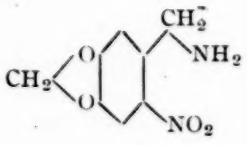


(VI)

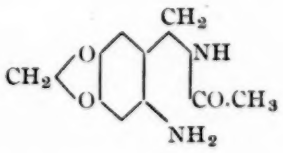


(VII)

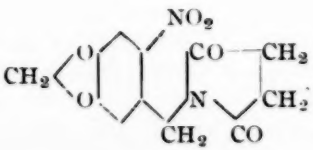
The substances of the type of (VI) in alcoholic solution easily cyclised to (VII) with a drop of potassium hydroxide solution at 50-60°. This general method of the formation of quinazolines has its limitations in that the acetyl derivative of 3:4-methylenedioxy-6-nitrobenzylamine (VIII) when reduced to (IX) did not pass into the corresponding quinazoline under all the conditions tried. The substance (IX) showed remarkable stability, whilst the analogous substance (X) presented no difficulty in its conversion to (XI).



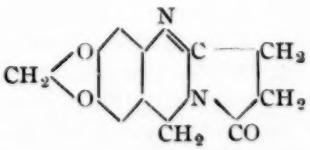
(VIII)



(IX)



(X)



(XI)

We can offer no explanation at this stage for the remarkable stability of (IX), but it is of so unusual a nature that we wish to draw attention to this peculiar behaviour.

EXPERIMENTAL.

o-Nitro-ω-piperidinoacetanilide (II, $n=1$, NR_2 replaced by piperidino).—A mixture of *o*-nitrochloroacetanilide (3 g), piperidine (3 c.c.) and benzene (20 c.c.) was heated on the steam-bath for 2 hours. The precipitated piperidine hydrochloride was filtered, the benzene solution washed with water and then the solvent removed. The pale yellow residue crystallised from hot petroleum ether in light yellow needles with slight greenish tinge, m.p. 83°. (Found: N, 15·97. $C_{13}H_{17}O_3N_3$ requires N, 15·79 per cent).

o-Amino-ω-piperidinoacetanilide.—The foregoing nitro compound (2 g.) dissolved in alcohol (12 c.c) was mixed with dilute hydrochloric acid (25 c.c. of 15%) so that no precipitation occurred. The mixture cooled to 0°, was reduced with zinc dust (15 g.) with small quantities at a time. The filtered solution was basified with ammonia in presence of ammonium chloride and the colourless oil extracted with chloroform, whence it was redissolved with dilute hydrochloric acid and reprecipitated with ammonia. After second precipitation, the oil solidified when cooled and scratched, yield 1.5 g. It crystallised from alcohol in colourless needles, m.p. 173° and gave a strong diazo reaction. (Found: C, 67·04; H, 8·28; N, 18·04. $C_{13}H_{19}ON_3$ requires C, 66·96; H, 8·16; N, 18·02 per cent).

Piperidinomethylbenziminazole (III, $n=1$, NR_2 = piperidino).—A mixture of the foregoing amine (0·5 g.) fused sodium acetate (1 g.), acetic acid (8 c c.) was refluxed for an hour. After dilution with water, the mixture was basified with ammonia when a colourless crystalline substance separated. It was crystallised from dry benzene in shining plates, m.p. 201°, yield 0·2 g. (Found: N, 19·52. $C_{13}H_{17}N_3$ requires N, 19·53 per cent). The urate of this base has a solubility of 0·105% in water at 20°.

o-Nitro-ω-diethylaminoacetanilide (II, $n = 1$, R = Et).—A mixture of *o*-nitrochloroacetanilide (3 g.), diethylamine (3 c.c.) and benzene (30 c.c.) was refluxed for 2 hours. The product, isolated as described before, crystallised from alcohol in yellowish needles, m.p. 70°. (Found: N, 16·83; $C_{12}H_{17}O_3N_3$ requires N, 16·74 per cent).

o-Amino-ω-diethylaminoacetanilide.—The nitro compound (2 g.) dissolved in alcohol (25 c.c) was added to a solution of ferrous sulphate (15 g.) in water (40 c.c.), basified with ammonia and kept at 80°. The

mixture was heated on the steam-bath for 20 minutes and then filtered hot. The filtrate was extracted with chloroform and the chloroform layer washed with dilute acid. A dark coloured impurity remained in the chloroform layer. The base was regenerated from the aqueous solution and was then taken up in ether. The dried ether solution furnished the amine on removal of the solvent. The substance was crystallised from hot petroleum ether in shining plates, m.p. 81°. (Found: N, 19·32. $C_{12}H_{19}ON^{[a]}$ requires N, 19·0 per cent).

α-Diethylaminomethylbenziminazole (III, $n = 1$, R = Et).— The cyclisation was effected as described before. The base was crystallised from a hot mixture of benzene and petroleum ether (1:1 in pale yellow plates, m p. 169°. (Found: N, 20·96. $C_{12}H_{16}N_3$ requires N, 20·60 per cent). The substance showed no tests for a free amino group whilst the amine, m.p. 81°, gave a brilliant scarlet azo-dye. The urate of the substance, m.p. 169°, had 0·42% solubility in water at 26°.

o-Nitro-β-chloropropionaniiide prepared in the usual way from *o*-nitroaniline and *β*-chloropropionyl chloride, had m.p. 85° after crystallisation from dilute alcohol. (Found: N, 12·45. $C_9H_9O_3N_2Cl$ requires N, 12·25 per cent).

o-Nitro-β-piperidinopropionanilide was prepared in the same manner as the corresponding acetanilide. The substance crystallised from hot petroleum ether in long greenish yellow needles, m.p. 44°. (Found : N, 15·0. $C_{14}H_{19}O_3N_3$ requires N, 15·16 per cent).

o-Amino-β-piperidinopropionanilide.—The above nitro compound (2 g.) in alcohol (15 c.c.) was reduced with zinc dust (15 g.) and hydrochloric acid (25 c.c. of 15%). The base was isolated in the manner previously described.

The colourless crystalline material isolated from the chloroform extract was recrystallised from boiling ligroin in fine needles, m.p. 110°. (Found: C, 68·0; H, 8·59; N, 16·84. $C_{12}H_{21}ON_3$ requires C, 68·0; H, 8·50; N, 17·0 per cent). The substance gave the test for an amino group.

The polymer of the Substance (IV).—The foregoing amine (1 g.), fused sodium acetate (4 g.) and acetic acid (25 c.c.) were refluxed for 1 hour. Thereafter, the mixture was diluted with cold water and basified with ammonia, when a colourless flocculent precipitate formed. This substance crystallised from alcohol in shining plates, m.p. 290° (decomp.), yield 0·5 g. [Found: C, 74·96; H, 4·87; N, 19·50. $(C_9H_8N_2)_n$ requires C, 75·0; H, 5·55; N, 19·46 per cent]. It is evident that the piperidine part has been knocked off during ring-closure resulting in an ethylene derivative. The high melting point points to the substance being a polymer. The substance

gave tests for unsaturation. Heating with zinc chloride, phosphoryl chloride or merely heating the amino compound at 200° results in the elimination of the piperidine residue with simultaneous ring-closure.

o-Nitro-β-diethylaminopropionanilide (II, $n = 2$, R = Et) prepared in the usual way was an oil which decomposed on distillation.

The related amine was prepared by reduction as follows :—A solution of stannous chloride dihydrate (7 g.) in acetic anhydride (7 c.c.) was diluted to 30 c.c. with glacial acetic acid. The solution was then saturated with hydrogen chloride at 0°. The foregoing nitro compound (2 g.) was added, with stirring to 60 c.c. of the ice-cold reagent prepared as above. After an hour, the separated tin double compound was filtered and then dissolved in a small quantity of water. The solution was basified with potassium hydroxide solution in the cold and the liberated base extracted with chloroform. The dried chloroform solution furnished the base on evaporation. It crystallised from hot petroleum ether in colourless needles, m.p. 56°. (Found : C, 66·56; H, 9·44. $C_{13}H_{21}ON_3$ requires C, 66·38; H, 8·94 per cent). This amine, on being heated with sodium acetate and acetic acid furnished the same substance as was obtained in the case of the corresponding piperidino compound. In this case diethylamine was extruded from the chain during ring-closure. The identity of this product with the polymer of (IV) described before was established by mixed m.p. and full elementary analysis. The identity of the products in the case of the *o*-amino-β-piperidino and the *o*-amino-β-diethylaminopropionanilide establishes fully the view that the basic part in either case is extruded during ring-closure.

The Quinazoline Series: o-ω-Chloroacetylaminobenzamide.—*o*-Aminobenzamide (2 g.) in benzene (20 c.c.) and pyridine (4 c.c.) was treated with chloroacetyl chloride (2 c.c.). The sticky brown solids were washed with water, dried, and crystallised from a large volume of benzene in light brown needles, m.p. 171°. (Found : N, 13·14. $C_9H_9O_2N_2Cl$ requires N, 13·18 per cent).

o-α-Piperidinoacetylaminobenzamide (VI, $n = 1$, R_2 = piperidino).—The above chloro compound (2 g.), piperidine (4 c.c.) in benzene (40 c.c.) was refluxed for 4 hours. After the removal of precipitated piperidine hydrochloride, the benzene solution furnished a dark product which was crystallised twice from hot benzene (charcoal) in light brown needles, m.p. 186° (decomp.), yield 1 g. (Found : N, 15·90. $C_{14}H_{19}O_2N_3$ requires N, 16·09 per cent).

2-Piperidinomethyl-4-oxyquinazoline (VII, $n = 1$, R_2 = piperidino).—A drop of potassium hydroxide solution was added to a solution of the

substance (0·5 g.) described in the preceding section in absolute alcohol (10 c.c.) and the mixture heated at 50-60° for 1 hour. After dilution with water (25 c.c.), the base was extracted with chloroform, the solution dried, and then the solvent removed. The residue crystallised from hot benzene—petroleum ether mixture (3:2) in fine colourless needles, m.p. 170°; yield 0·4 g. (Found: N, 17·54. $C_{14}H_{17}ON_3$ requires N, 17·28 per cent).

2-α-*Diethylaminomethyl-4-oxyquinazoline* (VII, $n=1$, R = Et).—o-ω-Diethylaminoacetylaminobenzamide was prepared as the corresponding piperidino compound with diethylamine. It was crystallised from a mixture of benzene-petroleum ether (1:1) in light yellowish needles, m. p. 165° (decomp.). After ring-closure with alkali, as described before, the substance crystallised from a mixture of benzene-petroleum ether (1:1), m.p. 85°. (Found: N, 17·85. $C_{13}H_{17}ON_3$ requires N, 18·18 per cent).

o-β-*Piperidinopropionylaminobenzamide* was prepared by interacting o-β-chloropropionylaminobenzamide and piperidine in the usual manner. The substance crystallises from benzene in colourless needles, m.p. 140°. (Found: N, 15·41 $C_{15}H_{21}O_2N_3$ requires N, 15·27 per cent).

2-β-*Piperidinoethyl-4-oxyquinazoline* was prepared by the action of a drop of alkali on the alcoholic solution of the foregoing substance at 50-60°. It crystallised from a mixture of benzene-petroleum ether in colourless needles, m.p. 148°. (Found: N, 16·47 $C_{15}H_{19}ON_3$ requires N, 16·34 per cent).

o-β-*Diethylaminopropionylaminobenzamide* (VI, $n = 2$, R = Et) had m.p. 99° after crystallisation from petroleum ether. (Found: N, 15·7. $C_{14}H_{21}O_2N_3$ requires N, 15·97 per cent).

2-β-*Diethylaminoethyl-4-oxyquinazoline* (VII, $n = 2$, R = Et) was prepared by the ring-closure of the above substance with alkali in the manner described before. The product crystallised from a mixture of petroleum ether (60%) and benzene (40%) in long needles, m.p. 122°. (Found: N, 17·10. $C_{14}H_{19}ON_3$ requires N, 17·14 per cent).

Piperonyl Alcohol. — 3 : 4 - Methylenedioxyphenylmethyl alcohol has been obtained in 90% yield by the electrolytic reduction of piperonal by Shima (*Mem. Coll,. Sci. Kyato*, 1928, (A), **11**, 407) but the following method is much easier to work and gives equally good yield.

Formalin (135 c.c.) was added to a solution of piperonal (100 g.) in methanol (120 c.c.) and the solution warmed to 60°. Sodium hydroxide (90 g.) dissolved in minimum amount of water was slowly added with constant shaking during 1/2 hour. The solution became dark during the addition of the alkali. After refluxing on the steam-bath for 1/2 hour

methanol was distilled off and the mixture diluted with water (300 c.c.) and then extracted with ether (400 c c.). The ethereal solution was dried and the solvent removed. The residue was distilled in *vacuo* and the distillate of piperonyl alcohol solidified to a colourless substance, m.p. 56°; yield 90 g.

3:4-Methylenedioxy-6-nitrobenzyl chloride was prepared by the excellent method of Robinson and Robinson (*J. Chem. Soc.*, 1915, **107**, 1758). We observed that after two-thirds of the nitric acid had been added, a sudden rise of temperature of the reaction mixture takes place. We found, however, that the initial addition of sulphuric acid to the reaction mixture after saturation with hydrogen chloride and before the addition of nitric acid, prevents this sudden reaction and the product crystallises quite easily in stout prisms from methanol, m.p. 86°.

In an experiment when piperonyl alcohol (90 g.) in acetic acid (100 c.c.) saturated with hydrogen chloride was nitrated with nitric acid (170 c.c., *d* 1·42) and sulphuric acid (5 c.c.) at 5-10°, 65 g. of the crystallised product were obtained.

N-3:4-*Methylenedioxy-6-nitrobenzylphthalimide*.— 6-Nitrohomopiperonyl chloride (5 g.), potassium phthalimide (4·25 g.), potassium iodide (1 g.) were mixed together and heated on the steam-bath for 20 minutes with absolute alcohol (15 c.c.). After cooling the precipitated solids were collected and well washed with water and then crystallised from hot acetic acid. It crystallised in pale yellow needles. m.p. 218°, yield 8 g. (Found: N, 8·58. $C_{16}H_{10}O_6N_2$ requires N, 8·5 per cent).

3:4-*Methylenedioxy-6-nitrobenzylamine* (VIII).—The foregoing compound (5 g.) suspended in alcohol (50 c.c.) was treated with hydrazine hydrate (*cf.* Ing and Manske, *J. Chem. Soc.*, 1926, 2348). The mixture was refluxed for 2 hours, when a thick gelatinous precipitate separated. At this stage, hydrochloric acid (*d* 1·16; 25 c.c.) was introduced and the heating continued for another 2 hours. The precipitate phthalyl hydrazide was filtered off, the filtrate evaporated to dryness on the steam-bath. The residue was lixiviated with water and filtered from a sticky insoluble solid. The aqueous solution on concentration gave the hydrochloride of 3:4-methylenedioxy-6-nitrobenzylamine which was recrystallised from ethyl alcohol, m.p. 196° (decomp.), yield 2·9 g. (Found: N, 14·46. $C_8H_8O_4N_2$,HCl requires N, 14·28 per cent).

The *free base* liberated with alkali was extracted with chloroform, whence a yellow solid was obtained. It crystallised from benzene in fine needles, m.p. 105° (decomp.). (Found: N, 13·75. $C_8H_8O_4N_2$ requires N, 14·28 per cent.). The base is moderately soluble in water,

alcohol, benzene and ethyl acetate. It is slowly oxidised when kept exposed to air.

3:4-*Methylenedioxy-6-nitroacetylbenzylamine* was prepared in the usual manner. After crystallisation from alcohol (pale brown needles) it had m.p. 204° (decomp.). (Found : N, 11·76. $C_{10}H_{10}O_5N_2$ requires N, 11·87 per cent.).

3:4-*Methylenedioxy-6-aminoacetylbenzylamine* (IX) was prepared by reducing the foregoing nitro compound (1 g.) with the stannous chloride reagent (30 c.c.) described before The tin double compound, dissolved in the least quantity of water was basified with potassium hydroxide solution. The *base* was extracted with chloroform, whence it came out as a semi-solid mass After repeated crystallisation from benzene, it came out in fine colourless needles, m.p. 126° (decomp), yield 0 4 g. (Found : C, 57·60 ; H, 6·01 ; N, 13·69. $C_{10}H_{12}O_3N_2$ requires C, 57 69 ; H,5 76 ; N, 13·46 per cent). The substance gave tests for an amine and resisted ring-closure with (*a*) sodium acetate and acetic acid, (*b*) acetic anhydride, (*c*) alkali in alcoholic solution and (*d*) phosphoryl chloride

N-3:4-*Methylenedioxy-6-nitrobenzylsuccinimide* (X) —A mixture of 6-nitropiperonyl chloride (5 g.), potassium succinimide (3·2 g), absolute alcohol (15 c.c.) was refluxed on the steam-bath for 3 hours. The separated solids were collected after cooling and after washing with water were crystallised from hot alcohol in light brown needles, m.p. 175°, yield 3 g. (Found : N, 10·27. $C_{12}H_{10}O_6N_2$ requires N, 10·07 per cent).

Methylenedioxyisovasicone (XI).—A solution of stannous chloride (5 g) in hydrochloric acid (*d* 1·16, 5 c.c) was warmed to 50° and small portions of the foregoing nitro compound (1 g.) added with stirring. After the addition of the whole amount, the temperature was raised to 60-70° for ½ hour The mixture was then diluted with water and strongly basified with potassium hydroxide solution in the cold. The liberated base was extracted with ethyl acetate, solvent removed, and then crystallised from hot alcohol in colourless needles, m p. 267°. (Found: C, 62·7 ; H, 4·5; N, 12·1. $C_{12}H_{10}O_3N_2$ requires C, 62·8; H. 4·3 ; N, 12·2 per cent).

All the analyses recorded above were done by the micro method.

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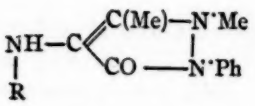
ACRIDINE DERIVATIVES AS ANTIMALARIALS. PART II.

By U. P. Basu and S. J. Das-Gupta.

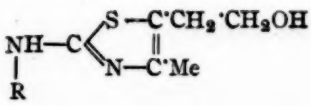
In view of the fact that 5-aminoacridine derivatives generally possess antimalarial activity, several such compounds have been prepared by condensing 2-chloro-7-methoxy-, 2-chloro-7-methyl-, 2 : 7-dichloro- and 3-nitro-7-methoxy-5-chloro-acridines with 4-amino-antipyrine, 2 amino-4-phenylthiazole and 2-amino-4-methyl-5-β-hydroxyethylthiazole (a ring present in vitamin B_1) respectively. All the compounds are coloured basic substances and form hydrochlorides readily soluble in water. As their molecular weights lie within the limit required for an antimalarial drug, they might be of some therapeutic importance.

Recent researches on the antimalarials point to the fact that for exerting an antimalarial activity the compound must contain a quinoline nucleus (acridine being regarded as a benzoquionoline) and be linked to a chain like dialkylamine, or to a complex like quinuclidine ring. That a mere linking with a chain like the former one, does not help the heterocyclic nucleus in this direction, is again evident from the observations of Slotta and Behnisch (*Ber.*, 1935, **68**, 754). It would now be a natural development to make a further study on the influence of other substituents on the above ring systems.

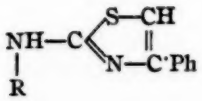
In the present paper certain 5-chloroacridines have been condensed with 4-aminoantipyrine, the dimethyl derivative (pyramidon) of which is known to exert a powerful antipyretic action. The resulting 4-acridylaminoantipyrins of the type (I) are generally reddish orange needles being readily soluble in dilute acids. Similarly, in view of the recent observations that vitamin B_1 contains a thiazole nucleus (4-methyl-5-β-hydroxyethylthiazole), the above chloroacridines have been fused with 2-amino-4-methyl-5 - β - hydroxyethylthiazole and 2 - amino - 4 - phenylthiazole to afford the corresponding acridylaminothiazoles (II) and (III) respectively. All these acridine derivatives are reddish crystalline substances and form hydrochlorides which are again readily soluble in water. The molecular weight of the compounds varies from about 400—450 and as these figures are found to lie within or near the limit required for an antimalarial drug (*cf.* Slotta and Behnisch, *loc. cit.*), it is being expected that they might be of some therapeutic importance.



(I)

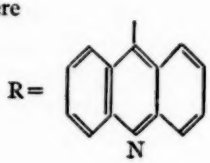


(II)



(III)

where



EXPERIMENTAL.

2-Chloro-7-methoxy-5-(1'-phenyl-2' : 3'-dimethyl-5'-pyrazolonyl)-aminoacridine.—2 : 5-Dichloro-7-methoxyacridine was prepared from 5-chloro-4'-methoxydiphenylamine-2-carboxylic acid and 1-phenyl-2 : 3-dimethyl-4-amino-5-pyrazolone which was obtained from antipyrine (*cf.* D. R. P., 71261). The dichloroacridine and the aminoantipyrine in equal parts were dissolved in phenol and heated on a boiling water-bath for 2 hours. The reaction mixture was cooled and poured into caustic soda solution (10%), when a solid separated out. This was filtered and washed free from alkali. On crystallisation from rectified spirit the compound was obtained in microscopic orange-yellow needles, m.p. 248°. (Found : N, 12·57. $C_{25}H_{21}O_2N_4Cl$ required N, 12·77 per cent). It is easily soluble in dilute acids and is reprecipitated on dilution with alkali.

2-Chloro-7-methyl-5-(1'-phenyl-2':3'-dimethyl-5'-pyrazolonyl)-aminoacridine.—The 5-chloroacridine derivative obtained from 5-chloro-4'-methyldiphenylamine-2-carboxylic acid, was condensed with 4-aminoantipyrine as in the previous case. The solid isolated was crystallised from rectified spirit in orange microscopic needles, m.p. 257°. (Found : N, 13·17. $C_{25}H_{21}ON_4Cl$ requires N, 13·07 per cent).

3-Nitro-7-methoxy-5-(1′-phenyl-2′ : 3′-dimethyl-5′-pyrazolonyl)-aminoacridine.—3-Nitro-7-methoxy-5-chloroacridine (m.p. 223°) obtained from 4-nitro-4′-methoxydiphenylamine-2-carboxylic acid, and 4-aminoantipyrine gave under the usual conditions a deep red product which on crystallisation from rectified spirit melted at 278-79°. (Found : N, 15·01. $C_{25}H_{21}O_4N_5$ requires N, 15·38 per cent).

2 : 7-Dichloro- 5- (1′-phenyl-2′:3′- dimethyl- 5′- pyrazolonyl)-aminoacridine.—2 : 5 : 7-Trichloroacridine, obtained from 4′ : 5-dichlorodiphenylamine-2-carboxylic acid, and the aminoantipyrine in equal proportions were heated together in presence of phenol for about 3 hours on a boiling water-bath. On cooling the mixture was diluted with ether when the hydrochloride of the condensation product separated out. This was filtered, washed with ether and finally treated with dilute ammonia. The residual mass was crystallised from dilute alcohol (1 : 1) and obtained in fine reddish needles, m.p. 276-77° (decomp.). (Found : N, 12·69. $C_{24}H_{18}ON_4Cl_2$ requires N, 12·47 per cent).

2-Chloro-7-methoxy-5- (4′-phenylthiazolyl-)—aminoacridine.—2 : 5-Dichloro-7-methoxy-acridine was dissolved in phenol on a boiling water-bath and treated with an equimolecular quantity of 2-amino-4-phenylthiazole (prepared from ω-bromoacetophenone and thiourea). The mixture was heated for more than 3 hours, when it was cooled and diluted with ether with stirring. A solid separated out which was collected, washed with ether and then triturated with dilute ammonia. The residual mass was filtered, washed with water and purified by repeated crystallisation from rectified spirit. (Found : Cl, 8·22. $C_{23}H_{16}ON_3ClS$ requires Cl, 8·5 per cent). It is a dark red crystalline substance, m.p. 246°-47° and is readily soluble in dilute acids.

2-Chloro-7-methyl-5-(4′-phenylthiazolyl)-aminoacridine.— Equimolecular quantities of 2 : 5-dichloro-7-methylacridine and 2-amino-4-phenylthiazole were heated in presence of phenol on an oil-bath at 120° for 3 hours. The reaction mixture on subsequent treatment as in the previous case afforded a red crystalline substance which separated from dilute alcohol (1 : 1) in fine microscopic needles, m.p. 263-64°. (Found : Cl, 8·97. $C_{23}H_{16}N_3ClS$ requires Cl, 8·84 per cent).

2:7-Dichloro-5-(4′-phenylthiazolyl) - aminoacridine.—2:5:7-Trichloroacridine and the aminophenylthiazole in molecular proportions were dissolved in phenol by warming and subsequently heated in on oil-bath at 120° for about 3 hours. The usual treatment of the reaction mixture yielded the condensation product which was subsequently crystallised from alcohol in

reddish needles, m.p. 269-70°. (Found: Cl, 16·97. $C_{22}H_{13}N_3ClS_2$ requires Cl, 16·82 per cent).

3-Nitro-7-methoxy-5-(4'-phenylthiazolyl)-aminoacridine.—3-Nitro-5-chloro-7-methoxyacridine was dissolved in phenol by heating on a water-bath. To the solution an equimolecular quantity of 2-amino-4-phenylthiazole was gradually added and the mixture was finally heated to 110-120° for about 3 hours on an oil-bath. After cooling, it was poured into caustic soda solution (10%) and the precipitate obtained was filtered and washed with water. It was then crystallised from alcohol in fine reddish needles, m.p. 264-65°. (Found: N, 12·88. $C_{23}H_{16}O_3N_4S$ requires N, 13·08 per cent).

2-Amino-4-methyl-5-β-hydroxyethylthiazole.—γ-Aceto-γ-chloropropyl alcohol (9 g.) was mixed with thiourea (6 g.) and absolute alcohol (30 c. c.) in a stoppered flask and left over at the room temperature (28°) for 2 days. A further quantity (3 g.) of thiourea was added and the mixture was refluxed on a steam-bath for 1 hour. Alcohol was then distilled off under reduced pressure and the residual mass was diluted with water when a clear solution was obtained. On saturating the solution with potassium carbonate an oily layer separated out. This was collected and directly distilled in *vacuo*. A yellow viscous liquid (3 g.) distilling at 200°/7 mm. was collected. It was dissolved in anhydrous ether and the ethereal solution was treated with dry hydrogen chloride. The hydrochloride separated out and was crystallised from a mixture of alcohol and ether and dried over sulphuric acid in *vacuo*, m.p. 138°. (Found: N, 14·13; Cl, 18·05. $C_6H_{10}ON_2S$, HCl requires N, 14·4; Cl, 18·25 per cent). The free base is moderately soluble in water and on long standing sets to a solid mass, m. p. 70°.

2-Chloro-7-methoxy-5-(4'-methyl-5'-β-hydroxyethylthiazolyl)-aminoacridine.—2:5-Dichloro-7-methoxyacridine (1 part) and 2-amino-4-methyl-5-β-hydroxyethylthiazole (1 part) in phenolic solution were heated together at 110-120° for 2 hours. The reaction mixture was poured over dilute caustic soda solution (10%) without allowing the temperature to rise. The solid was collected, dissolved in dilute acetic acid and filtered. The filtrate was made alkaline with dilute ammonia in cold when the acridine base separated out. This was collected, washed and crystallised from alcohol and water (1:2). (Found: Cl, 8·69. $C_{20}H_{18}O_2N_3ClS$ requires Cl, 8·88 per cent). It is a fine orange coloured solid, m.p. 256° (decomp.), soluble in common organic solvents and readily soluble in acids.

2-Chloro-7-methyl-5-(4'-methyl-5'-β-hydroxyethylthiazolyl)-aminoacridine.—2:5-Dichloro-7-methylacridine and the above thiazole in equal parts were heated in presence of phenol as usual. The reaction mixture was

poured into dilute caustic soda solution and the solid separating on being washed with water was crystallised from rectified spirit, m. p. 254°. (Found : Cl, 9·06. $C_{20}H_{18}ON_3$ ClS requires Cl, 9·25 per cent).

2 : 7-*Dichloro*-5- (4′-*methyl*-5′-*β*-*hydroxyethylthiazoiyl*) - *aminoacridine*.—2:5:7-Trichloroacridine and the thiazole afforded the condensation product under the usual conditions. It was crystallised from alcohol in fine orange microscopic needles, m.p. 273°. (Found : Cl, 17·44. $C_{19}H_{15}ON_3Cl_2S$ requires Cl, 17·58 per cent).

3-*Nitro* - 7 - *methoxy* - 5- (4′-*methyl*-5′-*β*-*hydroxyethylthiazoiyl*) - *aminoacridine*.—Equal quantities of 3-nitro-7-methoxy-5-chloroacridine and the hydroxyethylthiazole were heated together as in the previous cases, and the reaction mixture on usual treatment afforded the aminoacridine derivative. On crystallisation from rectified spirit it was obtained in reddish needles, m. p. 261-62° (decomp.). (Found : N, 13·96. $C_{20}H_{18}O_4N_4S$ requires N, 13·65 per cent).

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A NOTE ON THE USE OF ADSORPTION INDICATOR IN ACIDIMETRY AND ALKALIMETRY.

By Sachindra Nath Roy.

In two previous papers (*J. Indian Chem. Soc.*, 1936, **13**, 486 ; 1937, **14**, 120), the applicability of adsorption indicators in acidimetry and alkalimetry has been described where fluorescein was used as the adsorption indictor in conjunction with lead and tin salts. In the present communication attempts have been made to describe the use of fluorescein as an adsorption indicator along with a bismuth salt.

Traces of bismuth subnitrate are added to a solution of nitric acid (not less than $N/5$) and a few drops of fluorescein. The solution is titrated with sodium carbonate or caustic soda. The procedure is the same as in the cases previously described. The equivalent point is marked by a sudden change in colour of the solution from dull green to deep yellow (in case of Na_2CO_3) or reddish yellow (in case of NaOH). The method, however, has a limited application and can be used in titrating nitric acids of moderate dilutions, as bismuth salts are insoluble in very dilute acids. The following table gives an idea of the range within which the method may be used. Eosin may be used instead of fluorescein, in which case the colour changes from dull red to brilliant scarlet.

Approx. strength of acid or alkali.	Vol. of alkaline soln. reqd. for a known vol. of acid (Methyl orange).	Vol. of alkaline soln. for the same vol. acid. (Fluorescein).
$2N$	15·00 c.c.	15·00 c.c.
N	15·00	15·00
$N/2$	15·00	15·00
$N/5$	15·00	15·05

The mechanism of the reaction may be explained as in the previous cases, as the result of adsorption of the dye on the precipitate of bismuth salt, formed by the excess of alkali after the acid is neutralised.

My thanks are due to Prof. A. Maitra for his kind interest in this work.

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INFLUENCE OF NON-ELECTROLYTES ON THE CATHODE EFFICIENCY OF COPPER DEPOSITION.

By Shridhar Sarvottam Joshi, Dushyant Narasingasa Solanki and T. V. Subba Rao.

A quantitative study has been made of the deposition of copper from acidified copper sulphate solution in the presence of varying concentrations of the non-electrolytes: ethyl alcohol, methyl alcohol, glycerol and acetone. The increase of the cathode efficiency with the concentration of the non-electrolyte up to the limiting value has been attributed to the decreasing solvent action of the bath solution over the deposited copper. Evidence has also been adduced for the possible existence of the complex cation between the metallic copper ion and the non-electrolyte. The depression in the cathode efficiency, especially in the presence of large excess of acetone and methyl alcohol, has been attributed to an interaction between the medium and the conducting material.

The influence of addition agents on the electrolysis of aqueous copper sulphate has been observed in the electrolytic extraction and refinement of copper. The addition of free sulphuric acid was found to be essential to get satisfactory and concordant results with the deposition of copper as has been pointed out by Campbell and Dickson (*Proc. Phil. Soc. Glasgow*, 1900, **31**, 52), Ellwood and Spear (*Eighth Inter. Cong. Appl. Chem.*, 1912, **21**, 99) and others. Ellwood and Spear, from the study of the effects of inorganic addition agents in general, suggested that the function of such agents is to keep the copper in solution; moreover, the addition of these inorganic materials increases the conductivity of the electrolyte, which has got its own advantages as well as disadvantages. Emil Bose (*Chem. Ztg.*, 1902, **26**, 47), Gannon (*Proc. Roy. Soc.*, 1894, **A 55**, 66), Gray (*Phil. Mag.*, 1888, *v*, **25**, 183) and others studied the applicability of Faraday's law and concluded that slight deviations of the law were apparent, the departures being attributed to several disturbing factors, *viz.*,

(*a*) The existence of the cation in two forms, leading to a possible disturbing factor, due to the reaction, $M + M \ldots\ldots 2M$.

(*b*) Dissolution of the deposited copper due to a solvent action of the solution.

(*c*) Chemical corrosion brought about by dissolved oxygen. A bibliography of many of the investigations upon the deposition of copper in the

presence of gelatin and similar addition agents in general, will be found in an excellent paper of Frolich (*Trans. Amer. Electrochem. Soc.*, 1924, **46**, 67). Since then the important papers of Fuseya and co-workers (Fuseya and Murata, *ibid.*, 1926, **50**, 235 ; Fuseya and Nagano, *ibid.*, 1927, **52**, 249) and of Marie and Buffat (*J. chim. phys.*, 1927, **24**, 470), and Marie and Claudel (*Compt. rend.*, 1928, **187**, 170) have been published.

The possible rôle of these addition agents in general, has been investigated in a review by Blum (*Colloid Symposium Monograph*, 1921, **V**, 300) who suggests the following factors for cases in which the addition agent has actually been found in the cathode deposits: " (1) Co-discharge of colloid particles and metal ions, (2) discharge of complex ions containing the metal and the colloid, (3) adsorption of colloid upon the face of the metal deposit, or (4) mechanical inclusion in the deposit." Blum further states in regard to these various possibilities that " it is difficult from the meagre data available to suggest the relative probabilities of these processes.....". However, other workers have expressed preference for one or the other of the particular views outlined above. Thus Bancroft (*Trans. Amer. Electrochem. Soc.*, 1913, **23**, 266) tacitly assumes that adsorption accounts for the inclusion of the addition agent in the deposit; Fuseya and Murata (*loc. cit.*) postulated the existence of complex cations formed from the metal ion and addition agent and produce evidence to show that such complex ions are doubtless formed. The results of Marie and Buffat (*loc. cit.*) were further confirmed, recently, by Taft and Messmore (*J. Phys. Chem.*, 1931; **35**, 2585) who came to the conclusion that the deposited copper adsorbs gelatin on its surface and have established that complex ions are formed between Cu^{++} and gelatin and that electrochemical process occurring at the cathode is primarily a discharge of Cu^{++}. Datta and Dhar (*J. Amer. Chem. Soc.*, 1916, **38**, 1156), from the comparison of the copper coulometers containing varying quantities of cane sugar and of tartaric acid with silver coulometers, came to the conclusion that the mass of the deposit increases to a limiting value with the increasing content of the addition agent and is by no means restricted to the case of gelatin. Foerster and Siedel (*Z. anorg. Chem.*, 1897, **14**, 106) reported that in the electrolysis of copper sulphate solution, addition of alcohol gave more concordant results. These experiments however, were not carried out on a quantitave basis and it is not clear as to how the addition of alcohols and other non-electrolytes affects the the conditions of the bath. The authors, however, have supposed that " the alcohol decreases the concentration of the copper ions and therefore the solubility of the deposited copper in copper sulphate solution."

Richard, Collins and Heimrod (*Z. physikal. Chem.*, 1900, **32**, 321) considering the various factors which led to the deficits in the deposit, in agreement with the views of Foerster and Siedel (*loc. cit.*), suggested that the mean value of the atomic weight of copper should be 63·563, which is less than the value 63·604 obtained by purely chemical means.

The following experiments were carried out to investigate the apparent discrepancies in the cathodic copper deposits from those to be anticipated from Faraday's law and if possible to throw light on the influence of some non-electrolytes in improving the quality and the amount of deposit.

Experimental.

The chemicals employed $CuSO_4$, $5H_2O$, H_2SO_4, etc. were Merck's guaranteed reagents. Ethyl alcohol, methyl alcohol and acetone were freshly distilled before use. Pure glycerine was used. As it has already been shown by Gannon (*loc. cit.*). Campbell and Dickson (*loc. cit.*), Ellwood ann Spear (*loc. cit.*) and others that free sulphuric acid is beneficial, so a solution of the following composition as recommended by Oettel (*Chem. Ztg.*, 1893, **17**, 543) was prepared.

Copper sulphate crystals = 150 g.
Concentrated sulphuric acid (d 1·84) = 50 g.
Diluted to one litre with distilled water.

In the following experiments 175 c.c. of the same stock solution, diluted to 200 c.c. with the requisite amount of non-electrolyte and water, was used as the bath solution. The copper cathode ($5 \times 2 \times 0{\cdot}2$ cm.) used was given an initial thick and uniform deposit of copper. A steady current of 50 milliamps. obtained by using storage batteries (4·3 volts) and adjustable resistance was allowed to pass through the bath solution for a known interval of time, usually 1 hour. A precision milliammeter of Weston's model was used to read the current. The cathode at the end of the experiment was washed free from the electrolyte with distilled water thoroughly, then with rectified spirit to remove water and finally with ether to remove traces of alcohol. It was then thoroughly dried over the fan, allowed to cool and weighed. Each observation was repeated at least twice. These are recorded in Tables I-IV.

In Table I, vertical column 1 gives the concentration of alcohol added, expressed as a percentage by volume; columns 2 and 3 give respectively the weight of cathode before and after deposition; column 4 gives the weight of copper deposited; column 5 gives the theoretical or calculated

value for the deposit of copper in accordance with Faraday's law ; column 6 gives the time in hours ; column 7 gives the cathode efficiency *viz.*, the ratio of the weight of copper deposited (column 4) to the weight of copper calculated (column 5), expressed as a percentage. The results in Tables II-IV are recorded similarly.

Table I.

Electrolyte = Acidified $CuSO_4, 5H_2O = 13{\cdot}13\%$; $H_2SO_4 = 4{\cdot}4\%$. Non-electrolyte = Ethyl alcohol. Temp. $= 24 \pm 0{\cdot}2°$. Current = 50 m. amps.

1	2	3	4	5	6	7	
% EtOH.	Wt. of cathode Initial.	Final.	Wt. of copper Deposited.	Calc.	Duration.	Cathode efficiency.	
0 c.c.	31·7980 g.	31·8553 g.	0·0573 g.	0·0593 g.	1 hr.	96·63	96·63%
„	31·9132	31·9705	0·0573	„	1	96·63	
0·3	32·1142	32·1722	0·0580	„	1	97·81	97·81
„	32·1720	32·2300	0·0580	„	1	97·81	
0·5	32·3458	32·4042	0·0584	„	1	98·47	
„	32·4621	32·5207	0·0586	„	1	98·81	98·53
„	32·5207	32·5790	0·0583	„	1	98·31	
1·0	32·5791	32·6376	0·0585	„	1	98·65	98·73
„	32·9891	33·0477	0·0586	„	1	98·81	
2·0	33·3595	33·4184	0·0589	„	1	99·31	
„	33·4184	33·4768	0·0584	„	1	98·47	98·86
„	33·4768	33·5354	0·0586	„	1	98·81	
5·0	33·6520	33·7106	0·0586	„	1	98·81	98·98
„	34·0603	34·1191	0·0588	„	1	99·14	

TABLE II.

Electrolyte = Acidified $CuSO_4, 5H_2O = 13{\cdot}13\%$; $H_2SO_4 = 4{\cdot}4$ %. Non-electrolyte = Methyl alcohol. Temp. $= 24 \pm 0{\cdot}2°$. Current = 50 m. amps.

1	2	3	4	5	6	7	
% MeOH.	Wt. of cathode Initial.	Final.	Wt. of copper Deposited.	Calc.	Duration.	Cathode efficiency.	
0 c.c.	34·1343 g.	34·1916 g.	0·0573 g.	0·0593 g.	1 hr.	96·63	96·63%
„	34·1916	34·2489	0·0573	„	„	96·63	
1·0	34·3664	34·4245	0·0581	„	„	97·97	97·97
„	34·4245	34·4826	0·0581	„	„	97·97	
5·0	34·5406	34·5993	0·0587	„	„	98·97	98·81
„	34·5993	34·6578	0·0585	„	„	98·65	
10·0	34·6578	34·7161	0·0583	„	„		
„	34·7161	34·7744	0·0583	„	„	98·31	98·48
„	34·7746	34·8331	0·0585	„	„	98·65	
„	34·8909	34·9493	0.0584	„	„	98·47	
*20·0	34·9493	35·0069	0·0576	„	„	97·12	97·47
„	35·0070	35·0650	0·0580	„	„	97·81	

* In this case crystallisation of copper compound begins.

TABLE III.

Electrolyte = Acidified $CuSO_4, 5H_2O = 13{\cdot}13\%$; $H_2SO_4 = 4{\cdot}4\%$. Non-electrolyte = Acetone. Temp. $= 24 \pm 0{\cdot}2°$. Current = 50 m.amps.

1	2	3	4	5	6	7	
% Acetone.	Wt. of cathode Initial.	Final.	Wt. of copper Deposited.	Calc.	Duration.	Cathode efficiency.	
0 c.c.	34·1343 g.	34·1916 g.	0·0573 g.	0·0593 g.	1 hr.	96·63	96·63%
„	34·1916	34·2489	0·0573	„	„	96·63	
1·0	35·2180	35·3055	0·0875	0·0890	1·5	98·31	98·31
„	35·3054	35·3637	0·0583	0·0593	1	98·31	
5·0	35·3637	35·4219	0·0582	0·0593	„	98·13	98·13
„	35·5378	35·5959	0·0581	0·0593	„	97·97	
„	33·6544	35·7126	0·0582	„	„	98·13	
10·0	35·9143	35·9726	0·0583	„	„	98·31	98·31
„	35·9726	36·0309	0·0583	„	„	98·31	
* 20·0	36·0310	36·0889	0·0579	„	„	97·63	97·63
„	36·0889	36·1468	0·0579	„	„	97·63	

* In this case also separation of crystalline copper compound takes place.

TABLE IV.

Electrolyte = Acidified $CuSO_4, 5H_2O = 13{\cdot}13\%$; $H_2SO_4 = 4{\cdot}4\%$. Non-electrolyte = Glycerine. Temp. $= 24 \pm 0{\cdot}2°$. Current = 50 m. amps.

1	2	3	4	5	6	7	
% Glycerine.	Wt. of cathode Initial.	Final.	Wt. of copper Deposited.	Calc.	Duration.	Cathode efficiency.	
0 c.c.	34·1343 g.	34·1916 g.	0·0573 g.	0·0593 g.	1 hr.	96·63	96·63%
„	34·1916	34·2489	0·0573	„	„	96·63	
1·0	36·2044	36·2628	0·0584	„	„	98·47	98·47
„	36·2628	36·3212	0·0584	„	„	98·47	
5·0	36·3794	36·4382	0·0588	„	„	99·15	99·04
„	36·4380	36·4966	0·0586	„	„	98·81	
„	36·4966	36·6142	0·1176	0·1186	2	99·15	
10·0	36·6726	36·7315	0·0589	0·0593	1	99·31	99·23
„	36·7311	36·7899	0·0588	„	„	99·15	
20·0	36·8484	36·9073	0·0589	„	„	99·31	99·23
„	36·9073	36·9661	0·0588	„	„	99·15	

Curves in Fig. 1 show the variation of the cathode efficiency as a function of the percentage of ethyl alcohol and acetone ; curves in Fig. 2 refer, to the use of glycerine and methyl aicohol.

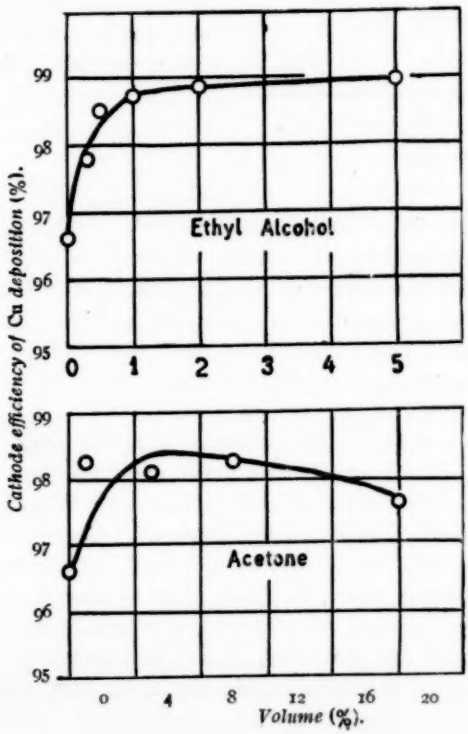
DISCUSSION.

Our data (*cf.* Tables I-IV) on the quantitative study of the deposition of copper from acidified copper sulphate solution in the presence of the varying concentrations of the non-electrolytes : ethyl alcohol, methyl alcohol, glycerine and acetone show that the cathode efficiency (*cf.* column 7, Tables I-IV) of copper inceases steadily with the progressive addition of the non-electrolyte, in all cases, till it approaches a limiting value ; it remains constant, being unaffected with further addition, in the case of glycerine (*cf.* Table IV, Fig. 2) but is depressed in the case of methyl alcohol (*cf.* Table II, Fig. 2) and acetone (*cf.* Table III, Fig. 1). The effect of the non-electrolytes studied, on the limiting value for the cathode efficiency is in the descending order : glycerine > ethyl alcohol > methyl alcohol > acetone, the limiting value being greatest in the presence of glycerine and least in the presence of acetone.

The apparent deficit in the copper deposit during the electrolysis of acidified copper sulphate solution, in absence of the non-electrolytes, can be attributed to solvent action on the deposited copper in the bath solution ; that the loss thus brought about, is a function of temperature and electrode

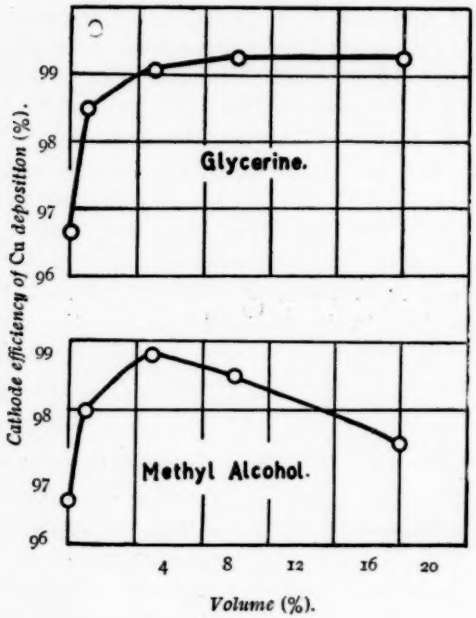
area of the cathode surface, is demonstrated by the work of Gray (*loc. cit.*), Foerster and Siedel (*loc. cit.*) and Richard, Collins and Heimrod (*loc. cit.*). Emil Bose (*loc. cit.*) attributed deviations from Faraday's law, when using salts, the cations of which can exist in two forms, to a possible disturbing factor, due to the reaction, $M+M.....2M$. Gannon (*loc. cit.*) has observed that copper sulphate in solution does not conform rigorously to the simple form, in which Faraday's law is generally expressed. The discharge of hydrogen, due to high current density is also another disturbing factor which gives too low a deposit. The addition of non-electrolytes, however, alters the condition of the bath and brings about the diminution in the concentration of the copper ions in the solution, thus lowering the solvent effect. It is, therefore, to be anticipated that an increase in the cathode efficiency would come about by increasing the concentration of the non-electrolyte up to a certain limiting value ; our foregoing results (*cf.* curves in Figs. 1-2) are in agreement with this deduction (*cf.* also Foerster and Siedel, *loc. cit.*).

FIG. 1.



In connection with the limiting value for the cathode efficiency, it is interesting to point out that with ethyl alcohol the efficiency steadily increases with the alcohol content up to 5%, and shows tendency towards the limiting value, which is not actually attained within the concentration range studied, unlike with the rest of the non-electrolytes, in which cases the limiting value is actually attained. The concentrations of the non-electrolytes corresponding to the limiting value for cathode efficiency are

FIG. 2.



found to be in simple molecular ratio with the molecular concentration of copper sulphate. The occurrence of maxima on the curves corresponding to the limiting value, may, possibly be attributed to the formation of a complex cation of the type $[\ Cu, n(\ CH_3OH\ )\ ]^{++}$, $[\ Cu, n(\ \text{glycerine}\ )\ ]^{++}$, etc. Such a possibility for the metallic copper ion to form a complex cation with the addition agent in general, has been shown, beyond doubt, to be valid in several cases as shown by several investigators : Fuseya and Murata (*loc. cit.*) in presence of addition agents in general; Fuseya and Nagano (*loc. cit.*) in the presence of glycocoll; Taft and Malm (*J. Phys. Chem.*, 1931, **35**, 874) in the presence of gum arabic; Taft and Messmore (*loc. cit.*) in the presence of gelatin, further confirmed from transport number measurements by Mutscheller (*Chem. Met. Eng.*, 1915, **13**, 353).

In spite of a general similarity of the behaviour shown by acetone it must be emphasised that acetone stands apart from the rest of the non-electrolytes investigated, *i.e.*, it does not contain (OH) hydroxyl group, so characteristic of the rest of the non-electrolytes employed. Capacity for forming a complex ion with copper is well known to depend, not only upon the colloidal nature of the addition agent, but also upon whether or not it contains hydroxyl or amino groups. The presence of a ketonic group is, therefore, anticipated to reduce this possibility, as is illustrated by the fact that addition of acetone raises the cathode efficiency to the least extent. Glycerine, probably due to its semi-colloidal and polyhydroxylic nature,

seems to be the most efficient in its action amongst the non-electrolytes studied. The limiting value in the descending order, glycerine > ethyl alcohol > methyl alcohol > acetone, might be attributed to a gradually diminishing tendency for complex formation as we pass from glycerine to acetone. Further evidence for a possible complex formation between the metal ion and the non-electrolyte has been adduced from the work carried out in these laboratories by Joshi and Padmanabhan (*This issue*, p. 176), the complex formation being suggested by inflexions on curves obtained by plotting transport number, viscosity, cathode efficiency, refractive index and conductivity against increasing concentrations of the non-electrolyte present in the system.

Our data with acetone and methyl alcohol show that cathode efficiency, after reaching the maximum, is reduced with further addition. This might be due to an interaction between the conducting material and the medium. In the presence of large excess of these non-electrolytes, due to limited solubility of the electrolyte in the medium, it is possible to imagine a stage of supersaturation, or even actual separation of the salt, a stage which was actually realised, especially in the presence of 20% non-electrolyte, as mentioned in the foot notes, in Tables II and III The separation of the salt naturally alters the concentration and thus upsets the conditions of the bath and this might be one of the possible factors responsible for the observed depression in the cathode efficiency.

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STUDIES OF SOME PHYSICOCHEMICAL FACTORS IN THE ELECTRODEPOSITION OF SILVER.

By Shridhar Sarvottam Joshi and S Padmanabhan.

Results are given for the optimum conditions for the production of a smooth and adherent deposit of silver in respect of the duration of electrolysis, the inter-electrode distance, the electrolyte concentration, temperature, current and the influence of addition agents such as gelatin, turpentine and carbon disulphide.

Detailed measurements are made of the cathode efficiency of silver in the presence of methyl alcohol, ethyl alcohol and acetone whose proportions were varied over a wide range. Curves cor-relating the above two quantities show marked discontinuities indicative of complex formations in the conducting medium. Data are also given for the conductivity, refractive index and density, (and for methyl alcohol) transport number and viscosity.

A review of the literature shows that the data for the electrodeposition of silver are restricted principally to the use of the double cyanide of silver. The following experiments were carried out in order to investigate the utilisability of dilute silver nitrate solutions, in respect of the following factors :

1. The optimum conditions for the production of a smooth and adherent deposit of silver at various values of the duration of the electrolysis, the inter-electrode distance, the electrolyte concentration, temperature and current.

2. Cathode efficiency for the silver deposition in the presence of varying amounts of methyl alcohol, ethyl alcohol and acetone.

3. Presence in small quantities of such materials as gelatin, turpentine and carbon disulphide.

It also became necessary for the interpretation of the results obtained to determine the specific conductivity, refractive index and density of the bath solutions under conditions described later.

Experimental.

The circuit consisted of an adjustable resistance, an accurate milliammeter and an electrolytic bath screened from light kept at constant temperature. Two lead accumulator cells of a large capacity were used as the source of the current. The electrodes used were rectangular pieces of pure

silver of the size, $1{\cdot}5'' \times 1'' \times 0{\cdot}05''$; they were suspended parallel from an ebonite piece, the distance being varied as required.

The electrodes were first cleaned with emery paper and then with dilute alkali to remove traces of grease. They were then dipped in a very dilute solution of nitric acid and rinsed with distilled water. A thin coating of silver was given to the cathode by passing a current of about 60 to 70 milliamperes for about half an hour in a cyanide bath. After cleaning as before, the cathode was washed finally with an alcohol-ether solution and dried over the fan.

The stock solution consisted of $N/5$-silver nitrate; it was prepared under red light and stored in red coloured bottles to minimise any light action. This was diluted to the required concentration in any given experiment. The precaution as to light action was also observed during electrolysis of the silver nitrate solutions used in this work. The following factors were studied in detail to investigate the optimum conditions under which a good adherent deposit of silver could be obtained, over ranges as indicated:

(*a*) Current, 4 to 50 milliamps.
(*b*) Concentration of electrolyte, $N/200$ to $N/20$.
(*c*) Inter-electrode distance, 0·5 to 5 cm.
(*d*) Temperature, 25° to 40°.
(*e*) Time, 30 to 120 minutes.

These experiments showed that the following factors represented the optimum conditions: (*a*) 7 milliamps., (*b*) $N/20$-$AgNO_3$, (*c*) 0·5 cm., (*d*) 26°, (*e*) 38 mins. Under these *optimum conditions* measurements were made of the cathode efficiency for the silver deposition, in the presence of (*i*) methyl and ethyl alcohol and acetone, their proportions in all the cases being 15, 25, 35, 40, 50, 60 and 75 % by volume of the bath solution, and (*ii*) agents introduced in traces, *viz.*, carbon disulphide, gelatin and turpentine. The nature of the silver deposit produced in each of these experiments is also recorded, as examined with a microscope.

The current and the duration of electrolysis were kept constant at 7 milliamp. and 30 minutes in all the experiments made for the determinations of the cathode efficiency. The weight of silver deposit as calculated from Faraday'a law for the above time is 0·01408 g. The cathode efficiency in any given experiment was given by the quantity, weight of the cathodic deposit of $Ag \times 100/0{\cdot}01408$. These results are reported in Table I, (*a*—*e*).

To obtain further information in regard to the results obtained for the influence of methyl and ethyl alcohol and acetone on the depositional

behaviour of the bath solution, the corresponding electrolytic conductivity was determined on a Kohlrauch bridge with the usual precautions. The cell constant determined by using $N/50$-KCl was found to be 0·3777. These results are given in 4th and 5th columns of Tables II to IV

The refractive index for the sodium line was determined for each of the above mixtures by means of Pulfrich refractometer at $26° \pm 0·1°$, with the usual precautions. The densities corresponding to these were determined at the above temperature. These results are given in Tables II to IV. In solutions containing methyl alcohol, measurements were made by Rao and Ramdas for the transport numbers and the corresponding viscosities. These have been shown graphically in Fig 3. Similarly the other quantities *viz.*, the cathode efficiency, refractive index, density and specific conductivity are plotted against percentages of the non-electrolyte added by appropriate selection of scale units in the same figure to economise space, in Figs. 1 to 3.

TABLE I.

(*a*) *Variation of current.*

Conc. $= N/20$-$AgNO_3$. Temp. $= 26°$. Inter-electrode distance $= 0·5$ cm. Time $= 30$ mins.

Current.	Nature of deposit.
50 m.amp.	'Tree' formation sets in as soon as electrolysis is started ; needle-shaped deposit.
40	'Tree' formation, slower ; size of needle-shaped crystals decreased.
30	'Tree' formation insensible ; deposit changes from needle-shaped crystals to big particles.
20	Particles decrease in size ; adherence partial.
10	Deposit irregular ; adherence better.
7	Deposit smooth, crystalline and adherent.
5	Deposit deteriorates.

(*b*) *Variation of concentration of electrolyte.*

Current $= 7$ m.amps. Time, inter-electrode distance and Temp. $= 26°$

Conc. of $AgNO_3$.	Nature of deposit.	Cathode efficiency.
$N/20$	Regular, adherent and smooth.	99·45
$N/100$	Less regular.	99·74
$N/200$	Black spongy needle-shaped crystals ; cathode turned dark.	

TABLE I (*contd.*).

(c) Variation of inter-electrode distance.

Conc. $= N/20\text{-}AgNO_3$. Temp. $= 26°$. Current $= 7$ m. amps. Time $= 30$ min.

Inter-electrode distance.	Nature of deposit.
5 cm.	Coarse, irregular and adherent.
4	More regular but coarse.
3	More regular and smooth.
2	More smooth than before.
0·5	Smooth regular deposit of good adherence.

(d) Variation of temperature.

Current $= 7$ m.amp. Time $= 30$ mins. Inter-electrode distance $= 0·5$ cm. Conc. $= N/20\text{-}AgNO_3$.

Temp.	Nature of deposit.	Cathode efficiency.
25°	Regular, adherent and smooth.	99·89
27°	No appreciable change.	99·45
40°	Do.	97·65

(e) Variation of time.

Current $= 7$ m. amps. Temp. $= 26°$. Inter-electrode distance $= 0·5$ cm. Conc. $= N/20\text{-}AgNO_3$.

Time.	Nature of deposit.	Cathdoe efficiency.
30 min.	Regular, adherent and smooth.	99·45
60	Do	99·74
90	Deposit partially adherent; size of crystals increased.	
120	Adherence poor.	

TABLE II.

Electrolyte = $N/20$-$AgNO_3$ soln. Non-electrolyte = Acetone. Temp. = $26 \pm 0.2°$.

% Acetone in $N/20$-$AgNO_3$ soln.	Cathode efficiency. Time = 30 min. Current = 7 m. amp. Dist. bet. the electrodes = 5mm		Sp. Conducty. Cell const. = 0.3777.		Refractive index		Density. Wt. of an equal vol. of water as soln at the same temp. = 9.9818 g. Density of water at 26° = 0.9968 g.	
	Wt. of deposit.	Cathode efficiency.	Resistance (ohms).	Sp. conducty (mhos).	Angle of prism.	Refractive index.	Wt. of soln.	Density.
0	0.0140 g.	99.5	246.6	0.00153	67°21′	1.332434	9.9926 g.	0.9979
15	0.0140	99.5	299.5	0.00126	66°1	1.338761	9.8350	0.9822
25	0.0131	93.1	337.7	0.00112	64°46	1.344906	9.7142	0.9701
35	0.01235	88.1	361.6	0.00104	63°57	1.349056	9.5940	0.9581
40	0.0120	85.3	403.3	0.00094	63°27	1.351619	9.5146	0.9501
50	0.0128	90.9	435.2	0.00087	62°41	1.355604	9.3330	0.9320
60	0.01325	94.5	484	0.00077	62°91	1.35842	9.1350	0.9122
75	0.0137	97.3	474	0.00080	61°11	1.363601	8.7824	0.8770

TABLE III.

Electrolyte-$N/20$-$AgNO_3$. Non-electrolyte = EtOH. Temp. = $26 \pm 0.2°$.

% EtOH in $N/20$-$AgNO_3$ soln.	Cathode efficiency. Time = 30 min. Current = 7 m. amp. Dist. bet. the electrodes = 5 mm.		Sp. Conducty. Cell const. = 0.3777		Refractive index.		Density. Wt. of an equal vol. of water as soln. at the same temp. = 9.9818 g. Density of water at 26° = 0.9968 g.	
	Wt. of deposit.	Cathode efficiency.	Resistance (ohms.)	Sp. conducty (mhos)	Angle of prism.	Refractive index.	Wt. of soln.	Density
0	0.0140 g.	99.5	246.6	0.00153	67°21′	1.332434	9.9926 g.	0.9979
15	0.0140	99.5	351.3	0.00107	65°54	1.339327	9.8224	0.9809
25	0.0136	96.6	434	0.00087	64°34	1.345929	9.6902	0.9677
35	0.0132	93.8	498	0.00076	63°40	1.350501	9.5744	0.9561
40	0.0122	86.7	532	0.00071	63°10	1.353081	9.4904	0.9477
50	0.0136	96.6	615	0.00061	62°23	1.357187	9.2720	0.9260
60	0.0140	99.5	684.5	0.00055	61°49	1.3602	9.0274	0.9016
75	0.0140	99.5	785.5	0.00080	61°25	1.362246	8.6724	0.8662

TABLE IV.

Electrolyte = $N/20$-$AgNO_3$. Non-electrolyte = MeOH. Temp. = $26 \pm 0.2°$.

% of MeOH in $N/20$ f. $AgNO_3$ soln.	Cathode efficiency. Time = 30 min. Current = 7 m.amps. Inter-electrode Dist. = 5mm.		Sp. Conductivity. Cell const. = 0·3777.		Refractive index.		Density	
	Wt of deposit.	Cathode efficiency.	Resistance (ohms).	Conducty. (mhos).	Angle of prism.	Refrac. index	Wt of soln.	Density
0	0·0140g	99·5	246·6	0·00153	67°21′	1·332434	9·9926	0·9979
15	0·0126	89·5	321·4	0·00117	66°45	1·335256	9·794	0·9781
25	0·0135	95·9	372	0·00101	66°19	1·337321	9·6632	0·9649
35	0·0140	99·5	432·3	0·00087	65°58	1·339003	9·5250	0·9510
40	0·0140	99·5	474·4	0·00080	65°49	1·339732	9·4560	0·9443
50	0·0136	96·6	536·6	0·00070	65°37	1·340707	9·2682	0·9255
60	0·01305	92·3	526·3	0·00072	65°39	1·340543	9·0758	0·9063
75	0·0122	86·7	457·6	0·00082	66°4	1·338521	8·7162	0·8704

TABLE V.

Electrolyte = $N/20$-$AgNO_3$. Currrent = 7 m. amp. Inter-electrode distance = 0·5 cm. Time = 30 min. Temp. = 26°.

% addition Agent.	Carbon disulphide.		Turpentine.		Gelatin.	
	Wt. of deposit.	Cathode efficiency.	Wt of deposit.	Cathode efficiency.	Wt of deposit.	Cathode efficiency.
0	0·0140 g.	99·5	0·0140 g.	99·5	0·0140 g.	99·5
1	0·0116	82·4	0·0108	76·7	0·0135	95·9
2	—	—	0·0086	61·1	0·0126	89·5
3	0·0090	63·9	0·0045	32·0	0·0122	86·7
5	0·0086	61·1	Black, needle shaped crystals; non-adherent deposit.		0·011	82·4
10	Cathode darkened. Deposit non-adherent.		—		—	—

Discussion.

The foregoing results show that the nature and the magnitude of an electrodeposit is conditoned by a wide variety of factors. These are discussed conveniently in respect of what has been designated as the cathode efficiency for the relevant ion, *viz.*, silver, which is 100% under ideal conditions. Any diminution in this appears to be due to such accessory circumstances as an incomplete adherence of deposit, occurrence of side reactions, *i.e.*, other than the main one for which the cathode efficiency is calculated. The discrepancy might also originate from solvational effects leading partly to the formation of molecular and ionic complexes in conducting mixtures. A number of experiments were carried out to investigate the influence of the factors designated *a*, *b*, *c*, *d*, and *e* (*vide*, p. 177) with a view to ascertain the optimum conditions for the relevant deposition and also in regard to the influence of (*f*) addition agents in small quantities and (*g*) proportion of a non-electrolyte varied over a wide range.

The process of electrodeposition is essentially a process of crystallisation. There are two stages in this process, *viz.*, (1) the formation of nuclei on the electrode surface and (2) growth of these crystal nuclei. Each of these has a different velocity. If the velocity of (1) is greater than that of (2), we get usually a fine deposit; whereas if the growth of crystal formation is more rapid, the deposit is coarser, and the big crystals are formed. The relation between the velocity of nuclei formation and the velocity of growth of crystals depends, besides the factors mentioned above *i.e.* (*a*) to (*g*), upon (*h*) nature of solvent, (*i*) nature of the metal deposited and (*j*) its valency.

Being of the '*nonpolarisable*' type, it is to be anticipated from general considerations that the deposit of silver obtained from a solution of silver nitrate will be in the form of projecting needles except under special conditions. The smooth surface obtained from the double cyanide of silver and potassium is attributed to the formation of complex ions.

A study of the results in Table I *b* shows that very dilute solutions of silver nitrate are not suitable for the electrodeposition of silver. For example, at the concentration $N/200$, the cathode is completely darkened as soon as the current is passed. This is in conformity with the results of the previous workers. Kohlschutter and Toropoff (*Z. Electrochem.*, 1923, **19**, 161) as a result of their investigations on the formation of silver black have emphasised that when a *very dilute* solution of silver nitrate is used, a black deposit is produced; it is due to the relative lack of crystal centres; under these conditions each discharged ion finds itself more or less isolated; consequently, it deposits itself as such and is not attached to a crystal centre, with the formation of grey crystalline silver. The sudden change to

grey silver on breaking the current is explained as an electro-surface tension phenomenon. The silver black with very large surface takes up the form of smallest surface on stopping the current, but when the current is flowing it is negatively polarised and the electrostatic repulsion of the charged particles overcomes the tendency to a surface diminution. Braun (*Ann. phys. chim.*, 1891, **42**, 450) has shown that silver is only deposited when the intensity of the current has a certain maximum value which depends upon the strength of the solution. Gray (*Phil. Mag.*, 1887, **22**, 389) has observed the marked value of a thorough cleaning of the electrodes and has given the size of cathode as ranging between 200 to 300 sq. cm./amp. current. He further adds that when the current is too small the deposited metal is not adherent and tends to grow out in crystals especially from any sharp edge or corner. An anode plate slightly larger than the cathode is recommended. Regarding the anode he is of opinion that it should have at least a surface of 400 sq. cm./ampere; very small anode plates become dark and spongy and gases are evolved from the surface, but when the plates are large they remain bright. Gray (*loc. cit.*) recommends a 5 % (10% at the most) solution of silver nitrate ; a silver anode, when not perfectly cleaned, is not completely dissolved; the outer layer of the metal remains as a loose skin. Barham and Taft (*J. Phys. Chem.*, 1930, **34**, 929) found that the deposits of silver obtained from silver nitrate in neutral, basic and acidic solutions were not good, were the poorest from neutral solutions, slightly better from basic and still better from acidic solutions. The deposits from neutral solutions were all badly burnt and in only a few cases, could be polished bright. Basic and acidic solutions also gave burnt deposits; they, however, in a number of cases took good polish. Barham and Taft (*loc. cit.*) from observations on variations of current density, found that above and below a certain critical value, the deposits distinctly deteriorate.

From results in Table I*e* it is seen that too long a prolongation of electrolysis leans to a decrease in the adhesiveness of the deposit. For example, after 90 minutes, it was seen that loose particles of silver got detached from the cathode, and remained suspended near it. The above-mentioned effect might be ascribed in part to one or both of the following factors : (1) The crystal structure tends to be loose due to an excess of duration of electrolysis, especially in the case of dilute baths, due partly to the impoverishment in the corresponding content of dissolved silver. Evidence in respect of this is afforded by the general experience that fresh baths deteriorate on long standing and continued electrolysis. Our results on the reduction in cathode efficiency by use of very dilute bath solutions are in agreement with the above suggestion. (2) Nitric acid produced near the

anode would diffuse and exert a solvent action on the cathode; it is expected that this would be more pronounced with longer duration of the electrolysis.

Results in Table I*d* show that at higher temperatures, the cathode efficiency of silver is decreased. This agrees with the results of Leduc (*Compt. rend.*, 1902, **135**, 237), who in the course of his investigations on the electrochemical equivalent of silver, found that nitric acid is not formed at the anode in appreciable quantities when normal solutions of silver nitrate are electrolysed at ordinary temperature the anodic density being lower than 0·002 C.G.S. units. Under these conditions the cathodic deposit of silver is quite normal. If the anodic density is greater, the concentration lower or *the temperature higher*, nitric acid is formed and disturbs the deposition of silver at the cathode, which means a decrease in cathode efficiency, as suggested by our experiments in Table I*d*.

Temperature affects the ionic velocity, degree of ionisation and the ionic mobility. In the case of copper sulphate a good deposit is obtained at higher temperature. This, however, does not hold good in the case of all metals as in the case of silver, where a decrease in cathode efficiency is noted (*cf.* Table I).

It is seen from results in Table I*a*, that high current densities tend to form needle-shaped crystals when silver nitrate solutions are used for electrolysis. This is in agreement with the results of previous workers. Hughes and Wethrow (*J. Amer. Chem. Soc.*, 1910, **32**, 1571) studied the effect of electrolytes on the electrodeposition of silver. In the case of nitric acid it was found that dense deposits could not be obtained if the E. M. F. was high, but with a low E. M. F. good crystalline deposits were produced. Samartzev (*Compt. rend. U. R. S. S.*, 1935, **2**, 478) showde that metallic deposits on the active portions of cathode were possible only at definite current densities.

The ratio between the current density and the concentration of the metal ion at the immediate vicinity of the cathode determines the nature of deposit. It is easily seen that local E. M. F. develops on account of the fact that the electrode is different from what obtains in the bulk of the system, after the commencement of electrolysis. By increasing the current density the rate of nuclei-formation is increased; if this is out of phase with the contemporaneous process of crystallisation, the deposit would be loose and spongy. When the rate of nuclei-formation and crystal growth are balanced, a smooth deposit results.

It is seen from Table I*c* that the quality of the deposit improves with the decrease in the inter-electrode distance. It has been shown already that

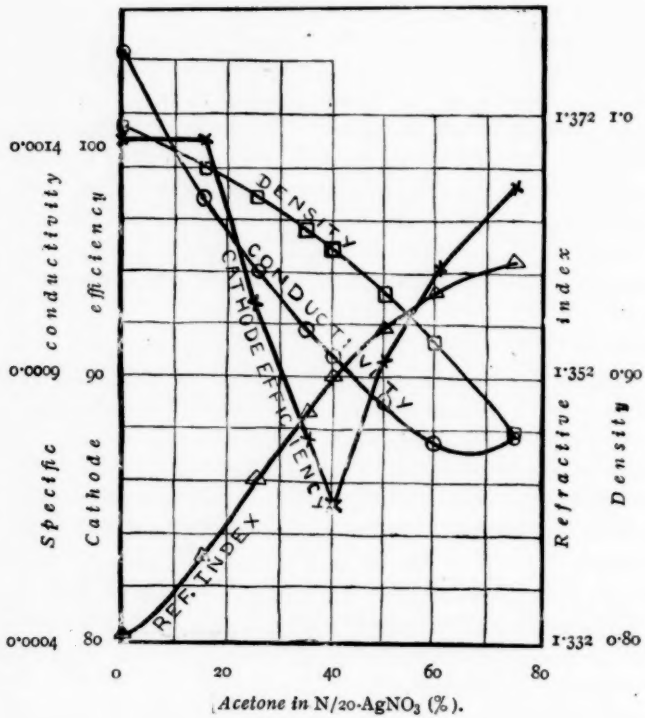
low current densities yield good deposits. A minimum of the foregoing quantities (7 m. amps and 0·5 cm.) represents the optimum conditions for silver deposition (*cf.* Table I *a* and *c*). Kremann (*Arhiv Hemiju*, 1930, **4**, 61) in the course of his study on the electrolysis of fused alloys produced by currents varied in the range 100-1000 amp./sq. cm. observed two determinants for the conductivity—one dependent on the fused state of the material and is presumably analogous to the so-called metallic conduction, and the other due to the electrolysis of the alloy, leading to changes in its composition in the vicinity of the electrodes, 'the metal of higher ionisation potential being deposited on the anode.' The latter effect becomes more marked as the current density increases and the distance between the electrodes is greater. These results suggest that a reduced inter-electrode distance would be the optimum condition corresponding to the use of low current densities. This deduction is in agreement with our results shown in Table I*c*.

No precise information is, however, available in the literature for the correlation of the cathode efficiency with proportion of an addition agent in the electrolytic bath (*cf.* results in Table V). We have studied this in the case of turpentine, carbon disulphide and gelatine whose concentrations were varied over a wide range. The magnitude of the above influence is illustrated by the fact that 1% of turpentine, for example, reduces the cathode efficiency by 17·04%. With smaller proportions of the addition agents, well below 1% of the deposit, is adherent, smooth and bright with sensibly normal values for the cathode efficiency. The deposits corresponding to higher proportions of the addition agents possess but poor structures consisting of loosely formed big crystals and in some cases the cathode is completely darkened.

Various theories attempting to explain the influence of addition agents, which are generally organic or colloidal substances, exist in the literature. From conductivity and transport number measurements, Wulff and Ehrenherg (*Phot. Korr.*, 1934, **70**, 155 ; *Chem. Zentrl.*, 1934, **II**, 3891) have shown that silver forms a complex ion with gelatin of the type $\{[Ag(Ge^-)_2]^-\}_n$. The apparently effective solubility of silver may be increased to about 100 times its normal value in water by the presence of gelatin. Frolich (*Trans. Amer. Electrochem. Soc.*, 1924, **46**, 67) found that gelatin has an 'isoelectric point' at p_H about 4·7 at which the material is electrolytically neutral and does not, therefore, migrate to either the anode or the cathode; at a p_H less than 4·7, *e.g.* in an acid copper solution, such colloids are positively charged and migrate towards the cathode surface and are actually included in the cathode deposits. Bancroft's view (*Trans.*

FIG. 1.

Temp. = 26 ± 0·2°.



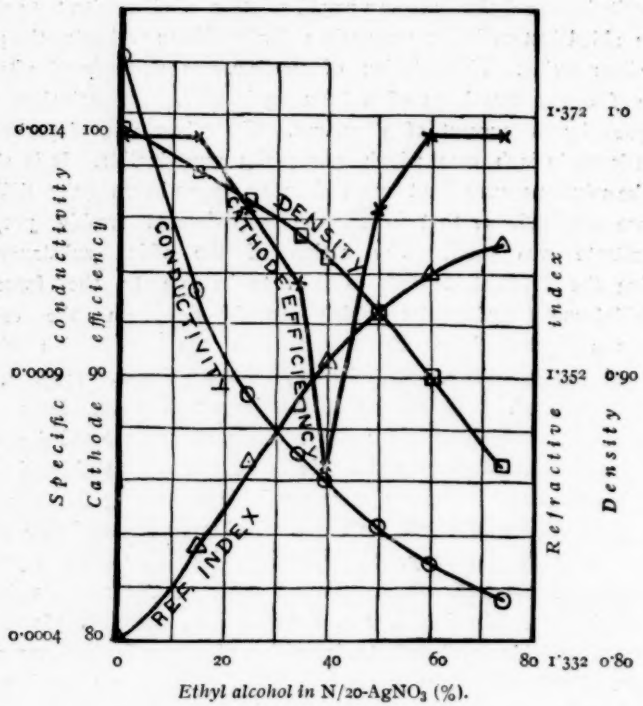
Amer. Electrochem. Soc., 1904, **6**, 27; 1913, **23**, 266) is that the crystal structure is decreased, when colloidal particles are thus adsorbed. It has been recently shown by Fuseya (*ibid.*, 1926, **50**, 235; 1927, **52**, 249) that substances such as citric and tartaric acids produce finer grained deposits of copper and silver from complex ions in the copper or silver solutions. It is possible that colloids may also form complex ions with the metals and be discharged on the cathode. Among the causes which normally promote the production of rough and 'treed' deposits is the presence of suspended impurities, especially conducting particles of carbon, metal, etc. derived from the anodes. Upthegrove and Baker (*Trans. Amer. Electrochem. Soc.*, 1928, **53**, 389) have observed that such impurities lodge themselves upon the cathode and serve as nuclei for the growth of large

grained crystals or '*trees*'. Much of the effect of addition agents in reducing roughness is that of counteracting the influence of suspended matter. Kohlschutter and Sachacht (*Z. Electrochem.*, 1913, **19**, 172) studied the influence of foreign substances on the deposition of silver. Silver was deposited electrolytically from a 0·05% solution of silver nitrate to which had been added just sufficient ammonium hydroxide to redissolve the precipitated silver oxide. This solution of 100 c.c. was electrolysed after the addition of one or two drops of sodium hydroxide. The solutions were electrolysed by a current of 3 m.amp. The silver was deposited in different forms in each case which was easily reproducible. It is shown that if the solutions were kept more than 15 to 20 hours quite different forms are obtained, so that in all the experiments freshly prepared silver nitrate was used. The action of the added substances in producing the different deposits is held to be due to the formation of colloidal metal hydroxides which are deposited on the cathode and form a type of filter, which conditions the distribution of the centres of crystallisation. Snowden (*J. Phys. Chem.*, 1905, **9**, 392) found that a good, coherent, crystalline deposit of silver from silver nitrate could be obtained by the rapid rotation of cathode and the separation of anodic and cathodic liquids. He is of the opinion that if means for a higher speed of rotation were available burnishing deposits would result. The addition of small quantities of glue had remarkable effects, smooth, bright, purple or yellow deposits with no visible crystalline structure being obtained which resemble some of Carry Lea's deposits from organic salts. Wernick (*Trans. Faraday Soc.*, 1928, **24**, 361) studied the effect of colloids on the electrodeposition of silver from silver nitrate solutions. He found that with the exception of dextrin there is a rough relationship between 'silver number' and the stiffness of deposit, the larger the 'silver number', the more closely grained was the deposit. Gorbunova (*Buil. Acad. Sci. U. R. S. S.*, 1933, 255) studied the structure of the electrolytic deposits of silver from its fused salts and found that they showed but feeble adherence and were readily removed by mere rubbing. Glazunov, Teindl and Halik (*Chem. Listy*, 1935, **29**, 117, 131) studied the influence of small amounts of agar-agar and gelatin on the velocity of crystallisation of cathode deposits of silver from aqueous silver nitrate and found that the above velocity is reduced by the presence of even 0·03 to 0·15% of agar-agar or gelatin.

It is to be anticipated that the complex formation as disclosed by the above results would be simpler when the addition agents were of molecular weights smaller than these characteristic of colloids. Experiments were

FIG. 2.

Temp. = 26 ± 0·2°.



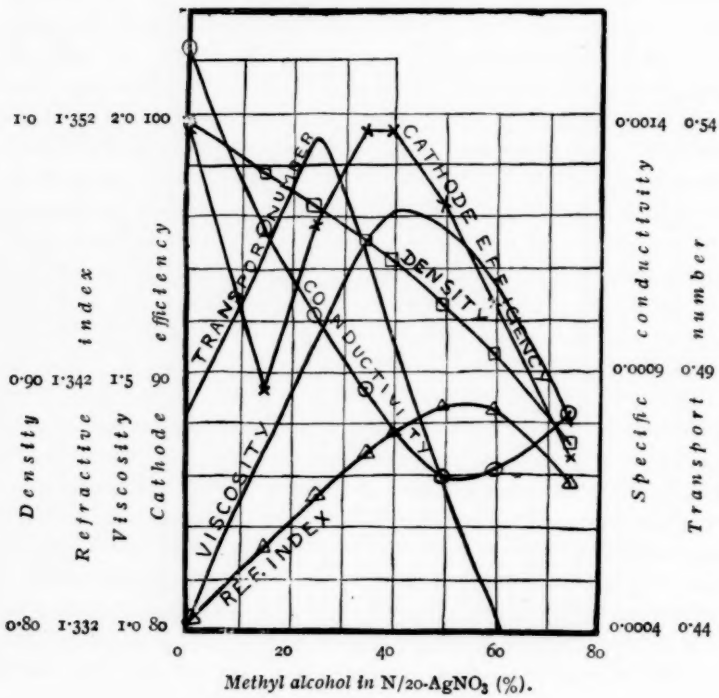
therefore, carried out on measurements of the cathode efficiency in the presence of varying proportion of acetone (Fig. 1 and Table II), ethyl alcohol (Fig. 2 and Table III), and methyl alcohol (Fig. 3 and Table IV). It is seen from these results that the above quantity is affected considerably due to the presence of the substances mentioned above. For example, in the case of acetone the cathode efficiency is almost constant at 99·45 % in the range 0 to 15% of acetone; it decreases very rapidly to a minimum at 40% and then increases continuously up to 75%. The influence of ethyl and methyl alcohol in respect of the above quantity (Fig. 2 and 3) is substantially analogous. It is extremely likely that these marked changes of gradient on the cathode efficiency—non-electrolyte concentration curves in Figs. 1-3 are due to some interaction between the medium and

the conducting material. A number of further properties such as the conductivity, the refractive index and density for the same mixtures were, therefore, studied. Schalls (*Z. physikal. Chem.*, 1894, **14**, 701) found that very usually, the conductivity is decreased when the water of solution is replaced in part by alcohol, which is in agreement with the foregoing results. Hantzuch (*Z. anorg. Chem.*, 1900, **25**, 332) studied the influence of non-electrolytes on the conductivity of electrolytes. The addition of methyl alcohol, ethyl alcohol and acetone to aqueous solutions of the alkali or alkaline earth metal diminished the conductivity slightly and approximately to the same extent. He also found that conductivity of ammoniacal solutions of calcium chloride is appreciably less than the sum of the conductivities considered separately. The difference is greater in the case of ammoniacal silver nitrate. The latter contains the complex ion $Ag(NH_3)_2$, so that the mobility of this ion might be about the same as that of the simple ion Ag. The results are here interpreted by Werner's theory. Jones and Rouiller (*J. Amer. Chem. Soc.*, 1906, **38**, 427), while studying this relative migration velocity of the ions of silver nitrate in water , methyl alcohol and ethyl alcohol and acetone, have studied the conductivity of such solutions. The conductivity was determined at 0° and 25°, all the measurements being made with 0·02*N* solutions. Observations confirmed that the velocity of the slower ion increases with the rise of temperature. This is not so in solutions in pure methyl alcohol or in a solution of methyl alcohol and water containing 75% of the former or in a mixture of methyl alcohol and acetone. The relative migration velocities are influenced largely by the nature of the solvent and this is probably due to the varying degrees of combination of the solvent with one of these ions.

The specific conductivity and refractive index graphs (Fig. 3) show a remarkable similarity, *i. e.*, while the specific conductivity decreases, refractive index increases gradually almost to the same extent until in the former case a minimum and in the later case a maximum are obtained. The corresponding curves in Figs. 2 and 3 show essentially the same feature. The occurrence of this discontinuity can, therefore, be taken as indicative of some complex formation. It is necessary to point out at this stage that although maxima and minima occur in all the curves showing the variation of the foregoing properties with the proportion of the non-electrolyte (except in the case of density where the discontinuity is markedly flatter) the actual position is not unique for all the curves. This would appear to be in accord with general experience of inflections on curves relating physical properties with chemical composition in various systems.

FIG. 3.

Temp. = 26 ± 0·2°.



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STUDIES IN VITAMIN C. PART V. THE VITAMIN C CONTENT OF SOME GERMINATED CEREALS AND PULSES.

By M. N. Rudra.

The vitamin C content of some germinated cereals and pulses has been determined by modified Tillmans method after reducing the trichloroacetic acid extract with H_2S. *Mung* has been found to synthesise the greatest amount (231·0 mg./100g. in 5 days) of vitamin C.

It is well known that germinated grains are good sources of vitamin C. Germinated Bengal gram and other pulses are often prescribed in cases of vitamin C deficiency. But for want of good quantitative data the dosage can only be decided upon empirically. Ghosh and Guha (*J. Indian Chem. Soc.*, 1935, **12**, 30) tried to estimate the vitamin C content of germinated *mung* titrimetrically but, when tested biologically, the germinated *mung* was found to be considerably richer in vitamin C content. The obvious explanation is that the ascorbic acid synthesised in germinated grains is mostly in the form of the reversibly oxidised dehydroascorbic acid (*cf.* Lee and Read, *J. Chinese Chem. Soc.*, 1936, **4**, 208) which is biologically active but cannot be estimated by the reduction of 2:6-dichlorophenol-indophenol. The present investigation was undertaken to make a quantitative assay of the vitamin C content of germinated grains so that they may be prescribed with a knowledge of the amounts of their vitamin C content.

Experimental.

Tap water was used for germination in all these experiments. The grains (50-200 in number depending upon their weights) were taken in a beaker, weighed and washed in several changes of water. They were then soaked in water for 24 hours. After this period the water was drained away and the swollen grains were allowed to germinate. If during germination, the grains became too much dried up, they were again moistened with a little water. The number of days of germination was calculated from the time they were kept under water for soaking. The vitamin C content of the germinated grains was then estimated by a slightly modified method of the author described in a previous paper (*Biochem. J.*, 1936, **30**, 701). The germinated grains (10-30 depending upon their weight) were taken and their original weight before being soaked was calculated. They were then

extracted with 20% trichloroacetic acid and through the filtrate a moderate current of hydrogen sulphide was passed for 15 minutes. The container was then stoppered, wrapped in a piece of brown paper and kept in the dark for 24 hours. Next day the extract was again filtered through a dry filter and through the filtrate a rapid current of carbon dioxide was passed till it was completely free from hydrogen sulphide as tested with moist lead acetate paper. It was now titrated against 2:6-dichlorophenol-indophenol solution which was standardised with ascorbic acid solution which in its turn was standardised with iodine solution. The titrations were timed with a stop-watch. The vitamin C content synthesised was then calculated on the basis of the original weights of the grains in the dry state before they were soaked in water. The results are given in Table I.

TABLE I.

Total vitamin C content of germinated grains.

Name of grains.	Period of germination.	Length of radicals.	Vitamin C content (mg./100g.)
Barley (*Hordeum vulgare*)	3 days	2-4 mm.	19·5
,,	5	6-10	31·1
Wheat (*Tritinum vulgare*)	3	1-5	17·3
,,	5	8-16	34·5
Bengal gram (*Cicer arietinum*)	2	2·5-5	40·7
,,	3	5-15	44·9
,,	4	10-20	50·6
,,	5	15-25	68·5
Mung (*Phascolus mungo*)	2	4-7	89·9
,,	3	6-14	123·5
,,	5	15-22	231·0

It thus appears that *mung* (green gram) can synthesise the largest amount of vitamin C, weight for weight, during germination. The cereals are capable of synthesising vitamin C to a lesser extent than the pulses. However, since the cereals can synthesise vitamin C to a considerable extent, it is of interest to find how much vitamin C, if any, is contained in malt extracts.

Malt is generally prepared by allowing barley and wheat to germinate for six days or more and then roasting the germinated grains and extracting

them. Unless all the vitamin C is destroyed during roasting, malt extract should be fairly abundant in vitamin C content. On examining a sample of concentrated extract of malt of a well known Calcutta firm, it was found that the total vitamin C content of the malt extract was 22·3 mg./100 g., whereas the vitamin C contents of wheat and barley after 5 days' germination only are 34·6 mg. and 31·1 mg. respectively. It would thus appear that some of the vitamin C is destroyed during the roasting process of the germinated grains in malting.

Discussion.

From the results it is apparent that the dicotyledons can synthesise relatively higher amounts of vitamin C than monocotyledons. As a result of the present investigation it would now be possible to prescribe germinated grains with a fair degree of accuracy about their vitamin C contents. It can be seen that 2 oz. of Bengal gram germinated for 2 days (23 mg. vitamin C) and 1 oz. of mung germinated for the same period (25·6 mg. vitamin C) would be sufficient to meet the daily vitamin C requirement of an adult. The relatively much higher vitamin C content of germinated mung raises the question if mung synthesises some other reducing substance besides ascorbic acid, especially as Johnson reports that germinated peas contain some other indophenol reagent-reducing substance than ascorbic acid (*Biochem. J.*, 1933, **27**, 1942). The apparently high vitamin C content of germinated mung may be due to some other factor which is under investigation (*cf.* Rudra, *Nature*, 1938, **141**, 203). Malt extracts contain a fair amount of vitamin C and, therefore, malt extracts combined with cod liver oil or halibut liver oil should contain the important vitamins A, B, C, and D. Manufacturers would do well to publish the vitamin C assay figures of their malt preparations along with the A, B, and D units content published by them. For similar considerations, malted whole milk should be comparatively richer in vitamin C content and would thus be more beneficial to scorbutic children than whole milk.

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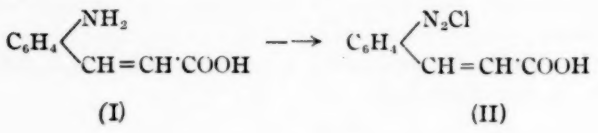
ISOMERIC TRIAZOCINNAMIC ACIDS AND RELATED COMPOUNDS.

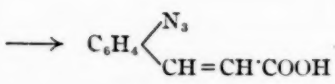
By K. Aswath Narain Rao and P. R. Venkataraman.

Nuclear substituted *triazocinnamic* and triazobenzoic acids have been prepared and their properties, with relative stabilities, investigated. The triazocinnamic acids give on oxidation the corresponding triazobenzoic acids and on reduction, different products depending on the agent employed With concentrated sulphuric acid, the triazocinnamic acids and *p*-triazobenzoic acid evolve two-thirds of the nitrogen content, the *o*- and *m*-triazobenzoic acids yielding a quantity of nitrogen which approximates more closely to one-half rather than two-thirds of the nitrogen content. F rmation of carbon monoxide, which is unusual, was noticed during these decompositions A striking difference in the comparative stabilities of the two series towards sulphuric acid of different strengths was brought to light by these experiments. The *para*-derivative is the least stable in the cinnamic acid series, whereas it is the most stable in the benzoic acids This is probably due to the different orienting influences of the original substituents in the two series. *Methyl α-triazocinnamate* has been prepared and its properties examined.

Aromatic triazo compounds have been subjected to many reactions involving decomposition, reduction and addition. In the present investigation, the nuclear substituted *triazocinnamic acids* and triazobenzoic acids have been prepared and their relative stabilities as well as other reactions studied. The triazocinnamic acids were prepared from the corresponding aminocinnamic acids by diazotisation and double decomposition with sodium azide, the amino-acids themselves being obtained from the nitro-acids by reduction.



(I) (II)

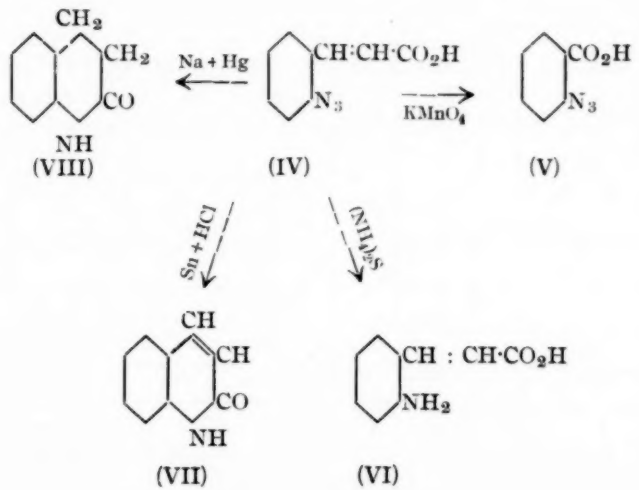


(III)

It is of interest to record the formation of a small quantity of an *acidic substance* decomposing at 268-70° during the preparation of *m*-triazocinnamic acid. The *o*-and *p*-nitrocinnamic acids were formed in almost equal

quantities by the nitration of cinnamic acid at 8-10° (*cf.* Müller, *Annalen*, 1882, **212**, 124) and were conveniently separated through their methyl esters, the ester of the *o*-nitro-acid being more soluble in methyl alcohol than the ester of the *p*-isomer. The triazobenzoic acids which have been prepared by Bamberger and de Werra (*Ber.*, 1902, **35**, 3718) by the interaction of the diazonium perbromides with ammonia, were more conveniently obtained by us from the appropriate aminobenzoic acids by diazotisation and interaction of the diazonium salt with sodium azide

A cold dilute solution of potassium permanganate converts the triazocinnamic acids into the corresponding triazobenzoic acids. The isomers behave differently, however, on reduction. The *m*-and *p*-acids on reduction with tin and hydrochloric acid or yellow ammonium sulphide give the corresponding aminocinnamic acids, while with sodium amalgam, aminohydrocinnamic acids are formed. The behaviour of *o*-triazocinnamic acid on reduction is slightly different. With ammonium sulphide, simple reduction of the triazo group occurs resulting in the formation of *o*-aminocinnamic acid (VI) and with tin and hydrochloric acid, carbostyril (VII) is formed, ring-closure taking place. On a more drastic reduction with sodium amalgam, dihydrocarbostyril (VIII) results, thus indicating that any of the three reduction products can be obtained by varying the reducing agent employed.



Hot 12 % potassium hydroxide has no appreciable action on *o*-and *m*-triazocinnamic acids, whereas in the case of the *p*-isomer, although a

part of the substance is recovered unchanged after 4 hours, there is produced a compound which does not melt or decompose up to 320°. It is known that aromatic triazo derivatives show a remarkable stability towards cold potassium hydroxide solution ; on the other hand, under similar conditions, the aliphatic as well as cyclic non-benzenoid triazo compounds decompose very rapidly (Forster and Fierz, *J. Chem. Soc.*, 1908, **93**, 73 ; Forster and Judd, *ibid.*, 1910, **97**, 255, 257). The decomposition of *p*-triazocinnamic acid is probably due to the well known influence of a *para*-orienting group on the *para*-substituent ; in this instance, the crotonyl group, $-CH=CH\cdot COOH$ makes the triazo group in the *para*-position unstable (*cf.* Forster and Fierz, *ibid.*, 1907, **91**, 1356). Resinification occurred when the triazocinnamic acids were boiled with 40% aqueous potash, the triazo group being destroyed in the process.

Triazo compounds on treatment with concentrated sulphuric acid are known to yield two-thirds of their nitrogen (*cf.* Forster and Müller, *J. Chem. Soc.*, 1910, **97**, 132, 137, 141). The three triazocinnamic acids and *p*-triazobenzoic acid also evolve two-thirds of the nitrogen content on being treated with strong sulphuric acid ; *o*-and *m*-triazobenzoic acids, however,

FIG. 1.

Triazocinnamic acids.

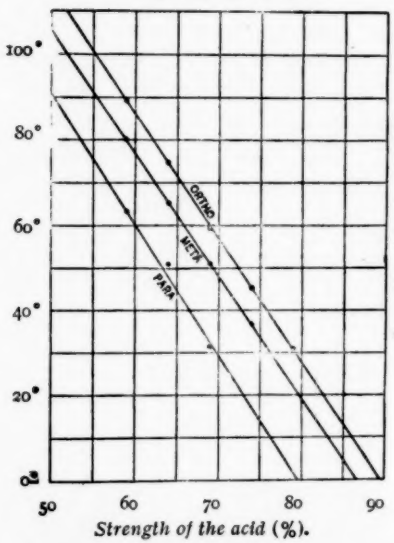
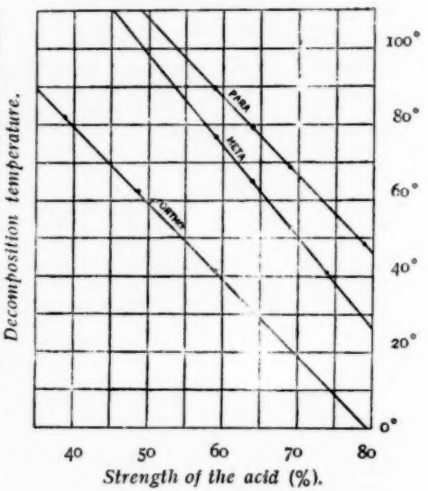


FIG. 2.

Triazobenzoic acids.



yield a smaller quantity of nitrogen, approximating more closely to half rather than two-thirds of the content of the element, the figures are not being sufficiently definite to admit of any general conclusion being drawn as to the course of the reaction. Such abnormalities have been observed in other cases also, especially by Forster and Fierz (*J. Chem. Soc.*, 1908, **93**, 79). An unusual feature of the decomposition of the triazo-acids used in the present investigation was the formation of carbon monoxide in addition to the usual products of decomposition, probably due to the presence of the unsaturated complex.

The comparative stabilities of isomeric triazocinnamic and triazobenzoic acids were studied by determining the temperature of decomposition with sulphuric acid of different strengths. The relation between the two was found to be a simple inverse linear function in all the cases as is evident from Figs. 1 and 2. It may be noted that the stability with reference to sulphuric acid increases from *para* to *ortho* in the triazocinnamic acid series, the *m*-isomer being intermediate ; the triazobenzoic acids, on the other hand, display surprisingly enough, a reverse order of stability, which increases from *ortho* to *para*, that of the *m*-isomer again occupying an intermediate position. It will be noticed that the *para*-derivative is the least stable in the cinnamic acid series, whereas it is the most stable in the benzoic acids. This is probably due to the different orienting influences of the original substituents in the two series ; the *para*-orienting group in the cinnamic acids makes the *para*-substituent unstable, while the carboxyl group is mainly *meta*-orienting in the benzoic acid series, although if this were the chief factor in determining stability, the *meta*-isomer should be the least stable in the latter case.

$C_6H_5.CH{=}CN_3{\cdot}COOH$ (IX)

$C_6H_5{\cdot}CN_3{=}CH{\cdot}COOH$ (X)

Attempts to prepare either α- (IX) or β- triazocinnamic acids (X) (Forster and Rao, *J. Chem. Soc.*, 1926, 1946) whose examination would be of interest for more than one reasons have not been successful. Not only would they contain a triazo group attached to a doubly linked carbon atom, but their possible conversion into α- or β-aminocinnamic acids would yield a rare class of substances. Replacement of the hydroxy group in α-triazo- β- hydroxy- β- phenylpropionic acid, $C_6H_5{\cdot}CHOH{\cdot}CHN_3{\cdot}COOH$ (XI) by a halogen

through the use of different halogenating reagents and subsequent removal of a molecule of hydrogen halide from the resulting substance did not meet with success. The compound (XI), which has been prepared by Forster and Saville (*J. Chem. Soc.*, 1922, **121**, 2598) by the interaction of cinnamic acid chlorohydrin, $C_6H_5{\cdot}CHOH{\cdot}CHCl{\cdot}COOH$ (XII) and sodium azide in aqueous solution was obtained in this instance by using the bromohydrin instead of the chlorohydrin. The former is obtained in better yield when prepared under conditions used by Forster and Saville (*loc. cit.*) for the preparation of (XII), than by the original method of Berner and Riiber (*Ber.*, 1921, **54**, 1954), considerable quantities of ω-bromostyrene being produced in the latter method, although the original authors do not record it. Synthesis of (IX) by the condensation of benzaldehyde with ethyl triazoacetate did not succeed.

On refluxing a mixture of cinnamic acid dibromide, in alcohol - pyridine and an aqueous solution of sodium azide, ω-bromostyrene and an acidic *substance* (m. p. 217°) containing nitrogen, but no triazo group was obtained. Owing to insufficiency of material, the constitution of this substance, whose dibromide melted at 176°, could not be determined. In view of the ease with which decarboxylation takes place when derivatives of the free acid are used, the action of sodium azide on the methyl ester of cinnamic acid dibromide was studied. An *oil* containing a triazo group as well as bromine was formed by this reaction, and gave on treatment with pyridine an unsaturated *ester* containing a triazo group. None of the liquids could be distilled without decomposition, even under reduced pressure. Although this ester could not be purified by distillation, it is clear from its properties and analysis that it is *methyl* α- (XIII) or β- triazocinnamate (XIV), probably the former.

$C_6H_5{\cdot}CH{=}CN_3{\cdot}COOMe$ (XIII) $\qquad$ $C_6H_5{\cdot}CN_3{=}CH{\cdot}COOMe$ (XIV)

EXPERIMENTAL.

Nitrocinnamic Acids.

The *o*- and *p*-nitro-acids were prepared by nitration of cinnamic acid at 8-10° and separated through their methyl esters. The *m*-nitro-acid was obtained by the condensation of *m*-nitrobenzaldehyde with malonic acid in presence of pyridine and piperidine.

Triazocinnamic Acids.

Aminocinnamic acid (54 g.), prepared by the reduction of the nitro-acid was stirred in a beaker surrounded by ice with water (108 c.c.) and 25% hydrochloric acid (74·5 c.c.). A solution of sodium nitrite (12·7 g. in 20 c.c. of water) cooled to 0° was added in the course of ten minutes with constant stirring. The diazotised solution was allowed to stand in ice for twenty minutes, then treated with enough urea to destroy excess of nitrous acid. An aqueous solution of sodium azide (13·7 g. in 108 c.c. of water) was then added dropwise with constant stirring, the temperature being maintained at 0°. Vigorous evolution of nitrogen took place, frothing being controlled by adding small quantities of ether from time to time After standing in ice for some time, the precipitated acid was filtered and purified, yield about 48 g. The dibromides were prepared by adding bromine in chloroform to a suspension of the triazo-acid in the same solvent. The triazo-acids do not detonate on heating but burn quietly with a vigorous flame. The *o*- and *p*-isomers display a tendency to decompose on keeping, characteristic of aromatic derivatives. The dibromides also exhibit a similar behaviour, the *m*-isomer being stable while the other two decompose on keeping.

o-*Triazocinnamic acid* crystallises from acetone (charcoal) in long needles melting at 186° (decomp.). (Found : N, 22·4; Equiv., 187. $C_9H_7O_2N_3$ requires N, 22·2 per cent. Equiv., 189). The acid is sparingly soluble in benzene but fairly soluble in chloroform. A solution of the acid in acetone exhibits green fluorescence. The acid and its solution in methyl alcohol are light brown in colour when freshly prepared, and darken on standing. While the acid was recovered unchanged after heating with 12 % potash on the boiling water-bath for 4 hours, it was converted into a resinous product containing nitrogen but no triazo group on refluxing with a 40% solution of potassium hydroxide during 4 hours. o-*Triazocinnamic acid dibromide* separates from benzene in long, prismatic needles, m. p. 165-66° (decomp.). (Found : Br, 45·7. $C_9H_7O_2N_3Br_2$ requires Br, 45 8 per cent).

Oxidised by a cold 4% aqueous solution of potassium permanganate in alkaline solution, *o*-triazocinnamic acid was converted into *o*-triazobenzoic acid, which after crystallisation from hot water melts at 143-44°. (Found : N, 25·6. Equiv., 164. $C_7H_5O_2N_3$ requires N, 25·8 per cent. Equiv., 163). *o*-Triazocinnamic acid on reduction with yellow ammonium sulphide in ammoniacal solution gives *o*-aminocinnamic acid, m.p. 158°, identified with an authentic specimen, while with tin and warm hydrochloric acid, carbostyril is formed, m.p. 199°. (Found : N, 9·8. Calc. for C_9H_7ON : N, 9·7

per cent). With sodium amalgam, however, dihydrocarbostyril melting at 163°, and identified by comparison with an authentic specimen, is produced. (Found : N, 9·7. Calc. for C_9H_9ON N, 9·5 per cent).

m-*Triazocinnamic acid* separates from acetone in prismatic needles, m.p. 165° (decomp.). (Found : N, 22·4 %. Equiv., 190). The *dibromide* crystallised from benzene, m. p. 157°. (Found : Br, 45·6 %). Towards potassium hydroxide *m*-triazocinnamic acid resembles the *o*-isomer. On oxidation with dilute permanganate, the *m*-triazo-acid is converted into *m*-triazobenzoic acid crystallising from water in glistening needles, m.p. 160°. (Found : N, 26·0 %. Equiv., 164). Freshly prepared yellow ammonium sulphide reduces *m*-triazocinnamic acid to *m*-aminocinnamic acid, m.p. 180°, formed also by reduction with tin and hydrochloric acid. With sodium amalgam, however, reduction of the ethylenic bond also takes place yielding *β-m*-aminophenylpropionic acid, which crystallises from water or alcohol in needles, melting at 83°. (Found : N, 8·7. $C_9H_{11}O_2N$ requires N, 8·5 per cent).

A small quantity of an acetone-insoluble material is always formed during the preparation of *m*-triazocinnamic acid. After purification by dissolving in pyridine, filtering and precipitating by acidifying, this *acid* crystallises from water in prisms decomposing at 268-70°. (Found : C, 65·1; H, 4·9; N, 8·7 per cent. Equiv., 98).

p-*Triazocinnamic acid*, crystallised from acetone, decomposes at 195-96° with slight decomposition at 160°. (Found : N, 22·5 %. Equiv., 188). The sodium salt is less soluble in water than the sodium salts of the *o*- and *m*-isomers. The *dibromide* crystallises from benzene in prisms, melting at 140-41°. (Found : Br, 45·5 %). It decomposes on keeping. On heating with 12% potash on a boiling water-bath for 4 hours, part of *p*-triazocinnamic acid is converted into a product which doos not melt or decompose up to 320°. This substance is insoluble in low-boiling solvents, but soluble in nitrobenzene. A dark resinous material is formed from the triazo-acid on boiling with 40% potassium hydroxide. Dilute permanganate oxidises the triazo-acid to *p*-triazobenzoic acid, which, crystallised from water, melts at 180°. (Found : N, 25·9 %. Equiv., 164). On reduction with yellow ammonium sulphide or tin and hydrochloric acid, *p*-triazocinnamic acid gives *p*-aminocinnamic acid melting at 175°, while with sodium amalgam *p*-aminohydrocinnamic acid crystallising from water in long

The equivalent weights of *m*- and *p*-triazocinnamic acids were determined by carrying out the titration in benzene solution, with frequent and thorough shaking, as the end-point was not sharp in methyl alcohol.

brownish needles, m.p. 131°, is formed. (Found : N, 8·6%). The acetyl derivative of this amino- acid melts at 142°.

Triazobenzoic Acids.

The hydrochloride of the aminobenzoic acid (20 g.) was mixed with a solution of 25% hydrochloric acid (26 c.c.) in water (100 c.c.) and diazotised with a solution of sodium nitrite (9 g. in 24 c.c. of water) at 0°. Enough urea was added after 20 minutes to destroy the excess of nitrous acid and a solution of sodium azide (9·2 g. in 25 c.c. of water) was added drop-wise. The precipitated triazobenzoic acid was filtered after 1 hour in the ice-bath and purified, yield 22 g.

o-*Triazobenzoic acid* crystallises from warm water in long needles melting at 143°-44°. (Found : N, 25·7; Equiv., 165. $C_7H_5O_2N_3$ requires N, 25·8 per cent. Equiv., 163). *m-Triazobenzoic acid* crystallises from aqueous alcohol in prisms, m.p. 160°. (Found : N, 25·5 per cent. Equiv., 164). p-*Triazobenzoic acid* separates from aqueous alcohol in prismatic needles, m.p. 180-81°. (Found : N, 26·5 per cent. Equiv., 164).

Decomposition of the Triazo Compounds by Sulphuric Acid.

(*a*) *Measurement of the Volume of Nitrogen evolved.*—The reaction was carried out in a bottle of 100 c.c. capacity and the gases generated were slowly allowed to bubble through a solution of cuprous chloride in hydrochloric acid to remove carbon monoxide and finally collected over 30% aqueous potash in a Lunge nitrometer. The volume of gas was read after standing over the alkali for 1 hour.

TABLE I.

Wt. of the substance.	Gas collected.	Pressure (mm. Hg)/T.	Vol. at N. T. P.	⅓ *N*, at N. T. P.
o-*Triazocinnamic acid.*				
0·0371g.	5·3 c.c.	764/27°	4·7 c.c.	4·4 c.c.
0·0699	10·3	,,	9·2	8·3
0·1045	21·4	,,	19·2	20·0
0·1890	26·8	,,	23·5	22·4

TABLE I (*contd.*).

Wt. of the substance.	Gas collected.	Pressure (mm. Hg) /T.	Vol. at N. T. P.	$\frac{1}{3}$ *N* at N. T. P.
m-*Triazocinnamic acid.*				
0·1332 g.	17·6 c.c.	763/27°	15·7 c.c.	15·8c.c.
0·1782	24·7	,,	22·6	21·2
0·1898	24·6	,,	21·9	22·5
0·2096	27·5	,,	24·6	24·6
p-*Triazocinnamic acid.*				
0·0412	5·6	761/29°	4·9	4·9
0·0838	10·5	,,	9·3	9·9
0·0844	10·7	,,	9·5	9·9
0·0883	11·8	,,	10·4	10·6
o-*Triazobenzoic acid.*				
0·0234	2·4	759/30·5°	2·1	3·2
0·0504	5·0	760/30°	4·4	6·9
0·0573	6·5	761/30	5·7	7·8
0·0588	7·3	759/30·5°	6·4	8·0
m-*Triazobenzoic acid.*				
0·0710	8·7	758·31°	7·6	9·7
0·0886	10·0	759/30·5°	9·2	12·1
0·0892	11·5	758/31°	10·0	12·2
0·1053	13·6	758/31°	11·9	14·4
p-*Triazobenzoic acid.*				
0·0376	5·7	762/29·5°	5·0	5·1
0·0428	6·8	,,	6·0	5·9
0·0536	8·0	,	7·1	7·3
0·0820	12·4	,,	11·0	11·2

(*b*) *Relation between Temperature of Decomposition and Concentration of Sulphuric Acid.*—For each experiment, 0·1 g. of the triazo-acid, finely powdered to pass through a 40 mesh gauze, was treated with 2 c. c. of sulphuric acid in a test-tube surrounded by two vessels with efficient

stirring arrangements to keep the temperature constant. The temperature of decomposition was noted when frothing started and gas wasevolved. The temperature recorded in Table II is the mean of three experiments.

TABLE II.

Strength of acid wt. per cent.	Decomposition temperature *ortho.*	*meta.*	*para.*
	Triazocinnamic Acid.		
59	88·8°	79·8°	62·9°
64	74·5°	65·0°	50·5°
69	59·0°	50·5°	30·9°
74	44·9°	36·6°	Room temp.
79	30·5°	Rapid decomp. at room temp.	Rapid decomp. at room temp.
84	Rapid decomp. in the cold.		
	Triazobenzoic Acid.		
39	82·1°	—	—
49	62·5°	—	—
59	41·5°	76·7°	89·7°
64	31·0°	64·6°	79·0°
69	Action in the cold, no charring.	52·5°	68·5°
74	,,	40·6°	58·5°
79	Vigorous action, dark green soln. formed.	Slow action	48·4°
84	,,	Immediate action	Vigorous reaction

Cinnamic bromohydrin (m. p. 125°), prepared by the interaction of solutions of sodium cinnamate and sodium hypobromite essentially under conditions used by Forster and Saville (*loc. cit.*) in the case of the chlorohydrin, was converted into α-triazo-β-hydroxy-β-phenylpropionic acid (XI), m. p. 121°, by interaction with sodium azide in alkaline solution. While hot 48% hydrobromic acid had no action on the triazohydrin, 25% hydrochloric acid converted it into a tarry substance when boiled. There was considerable decomposition with thionyl chloride or phosphorus pentachloride or pentabromide.

Action of Sodium Azide on Cinnamic Acid Dibromide in presence of Pyridine.—A mixture of the finely powdered dibromide (10 g.), ethyl alcohol (55 c. c.) containing pyridine (5 c. c.) and a solution of sodium azide (2·8 g. in 20 c. c. of water) was refluxed for 3 hours. The residue after removing the major portion of the alcohol was treated with excess of dilute hydrochloric acid and the solid which separated on standing, was filtered, washed with a little petroleum ether after draining, pressed on a porous plate and crystallised from hot water when it separated in fine, hairy needles, m.p. 217°. (Found : C, 60·6 ; H, 4·0 ; N, 8·0%. Equiv., 144). It decolourises potassium permanganate and bromine water. Its *dibromide* crystallises from alcohol in prisms, m. p. 176° with previous softening at 171°. (Found : Br, 49·4%). From the mother liquor of the acid, small quantity of ω-bromostyrene could be obtained.

Action of Sodium Azide on Methyl αβ-Dibromo-β-phenylpropionate.—A mixture of the ester (20·6 g.), methyl alcohol (68 c. c.) and an aqueous solution of sodium azide (8 g. in 25 c. c.) was refluxed on the steam-bath for 14 hours, the original turbidity disappearing in 3 hours. The product obtained by the removal of most of the alcohol, was diluted with water, the solution saturated with common salt and the precipitated oil extracted with ether thrice. The combined ether extracts after drying over anhydrous calcium chloride and removal of solvent gave a pale, clear orange-coloured oil (12 g.) which produced effervescence with concentrated sulphuric acid and contained bromine. The oil (11 g.) was mixed with pyridine (8 c. c.) and heated on the water-bath. After 3 hours, during which period some pyridine hydrobromide had separated, the reaction mixture was cooled, acidified with dilute hydrochloric acid and the solution extracted with ether. On removing the solvent from the washed and dried ether extract an *oil* was obtained which by its properties and analysis can be *methyl* α- or *β-triazocinnamate* (XIII) or (XIV). (Found for the undistilled specimen after standing in the vacuum desiccator for some weeks : N, 21·6. $C_{10}H_9O_2N_3$ requires N, 20·71 per cent). The ester decolourises neutral potassium permanganate and bromine water and vigorously effervesces with concentrated sulphuric acid ; but a dibromide could not be obtained. On oxidation with permanganate, partial conversion to benzoic acid occurred, indicating the position of the triazo group to be probably α.

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THIOKETONIC ESTERS. PART VII. THIO-THIOL ESTIMATION.

By S. K. Mitra.

A method for the estimation of the thio-thiol has been described. Substitution at the α-carbon atom of β-thioketonic esters increases the percentage of thiol. The factors governing this equilibrium have been clearly established The existence of a third phase—an enol, isomer of these esters has been suggested.

The orthodox bromination method for keto-enol estimation (Meyer, *Annalen*, 1911, **380**, 212) could not be suitably used in the case of β-thioketonic esters due to following reasons:

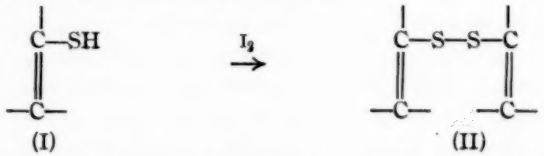
1. Various reactions were likely to occur, over and above the addition of bromine at the double bond, *e.g.* (*a*) oxidation of thiol to disulphide; (*b*) further oxidation to sulphone; (*c*) addition of bromine to the sulphide.

2. Part of bromine might react with the reactive hydrogen atom of the methylene group.

3. Addition to the double bond might not be quantitative.

It was thought that alcoholic iodide could be suitably used in determining thiol in such compounds.

The process underlying this method invokes the property of iodine to oxidise a thiol (I) to a disulphide (II).



(I) (II)

The result of the experiments have been described in Table I. A rise of temperature depresses thiolisation, which is in conformity with the effect on oxygen analogue (*loc. cit.*).

TABLE I.

Esters.	The percentage of thiol in EtOH at 30°.	40°.	60°.
$Me{\cdot}CS{\cdot}CH_2{\cdot}CO_2Et$	41·0	—	38·7
$Me{\cdot}CS{\cdot}CHMe{\cdot}CO_2Et$	62·8	61·9	60·0
$Me{\cdot}CS{\cdot}CH(CH_2{\cdot}CH{\cdot}Me_2){\cdot}CO_2Et$	—	—	64·4
$Me{\cdot}CS{\cdot}CH{\cdot}(CO_2Et)_2$	61·1	—	58·5

At ordinary temperature, ethyl thioacetoacetate contains nearly six times as much thiol as the enol in ethyl acetoacetate under similar conditions. Elhyl methylthioacetoacetate on the other hand contains a higher percentage of thiol than ethyl thioacetoacetate and this is the opposite to what was expected from the relation of ethyl methylacetoacetate and ethyl acetoacetate (*loc. cit*) though in each case rise of temperature favours thioketonisation. It is also evident that substitution in ethyl thioacetoacetate increases the percentage of thiol. The order is represented as

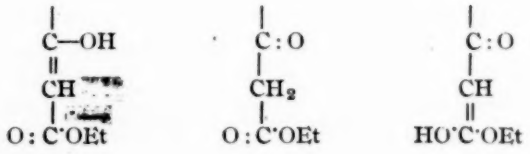
*iso*Butyl > Methyl > Carbethoxy > Hydrogen

The paradox that an increase of thiol due to the introduction of an electron pushing group clearly points out the existence of some condition other than the activation of the methylene group in the immediate neighbourhood of thiocarbonyl group, which controls the equilibrium.

There are certain activating goups (Lapworth, *Lit. Phil. Soc. Manchester*, 1920, **64**, *iii*, 1) which by virtue of their tendency to repel or attract electrons from a system acting in the form of electron source in the former and electron sink in the latter (Ingold, *J. Chem. Soc.*, 1926, 1305) create an increased tendency for an atom or a group in the system to separate as a positive or a negative ion, to combine with other external polarised molecules in a reaction. In the case of the thioketonic esters both C=S and −COOEt act as an electron sink and thus increase the tendency of the hydrogen atom of the methylene group to part as a positive ion. An altogether different behaviour *viz*., mobility of this hydrogen atom to that of methane is evidently due to this incipient ionised condition, which used to be known as active state. Such activation is necessary to endow the hydrogen atom with a tendency to react with an unsatuarated system either intermolecularly—aldol condensation, Michael's and Thorpe's reactions—or intramolecularly—triad and keto-enol tautomerism. Keto-enol tautomerism is, evidently a manifestation of an internal additive process—direct and retrograde, if it is, as suggested by Ingold (*Chem. Ind.*, 1923, **42**, 1246), considered to be an internal aldol condensation. The duty of the activating group is finished by imparting the required mobility to the hydrogen atom; it is the unsatuaration of the groups in tautomerides −C=O, C=C, in β ketonic esters and β diketones; C=S, C=C in corresponding sulphur analogues which control the distribution. Hence the tendency of saturation of these groups is the main factor governing the equilibrium. It is not difficult to imagine a case where

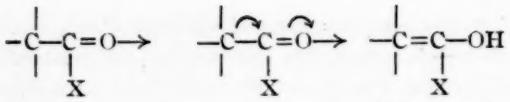
two unsaturated groups, *e.g.*, C=O and C=S with C=C, are competing for a hydrogen atom—the greater the unsaturation, the greater the share of hydrogen—a phenomenon though not identical but comparable with the distribution of a base between two acids. The greater percentage of thiol in ethyl thioacetoacetate than enol in the oxygen analogue is evidently due to the greater unsaturated character of the thiocarbonyl group. The increased thiolisation resulting from the substitution at the α-carbon atom in ethyl thioacetoacetate is due to the weakening of the unsaturated character of the C=C group in the substituted system. For a similar reason the carbonyl group of aldehydes is more reactive than that of ketones.

The almost equal percentage of enol in ethyl acetoacetate and its methyl derivative as determined by Meyer's method (if the method be taken for granted as correct) can be accounted for if β-ketonic esters be assumed to exist in three phases—A, B and C—at any time.



(A) (C) (B)

Meyer's method of bromination gives an estimation of the sum total of the phases (A) and (B). The introduction of a methyl group at the α- carbon atom evidently depresses the phase (B) by an amount almost equal to the increase of phase (A). The possibility of the existence of phase (B) was first suggested by Lapworth (*J. Chem. Soc.*, 1904, **85**, 30). According to him, initial enolisation is necessary for a ketone or an ester to react with bromine giving rise to α-bromo derivatives. The view has been confirmed by Ward (*J. Chem. Soc.*, 1923, **123**, 2212) who isolated intermediate compounds in some cases. Burton and Dawson (*J. Chem. Soc.*, 1914, **105**, 1289) have been able to confirm that substitution is preceded by isomeric transformation of an aldehyde from the ketonic to the enolic form. Meyer (*Ber.*, 1922, **55**, 2864) has also pointed out that enolisation of malonic acid is necessary before actual bromination takes place. This view has been confirmed by West (*J. Chem. Soc.*, 1924, **125**, 1277). According to the hypothesis of electronic displacement, any carboxylic compound (III) has a tendency to pass on to (IV).



(III) (IV)

Augmentation or retardation depends upon the nature of group 'X', where X is hydroxyl, as in the case of acetic acid ; there will be a little tendency for a proton to become loosened from the methyl group, since the carboxyl group is self-contained (Robinson, *J. Chem. Soc.*, 1925, **127**, 1618). No such adjustment of co-valencies of the carbonyl carbon is possible when 'X' is halogen, alkyl or hydrogen and hence loosening of a proton in these cases will occur with greater facility. The loosened proton is attracted towards negative carbonyl oxygen and enolisation takes place. In the case of β-ketonic esters, inspite of the impeding nature of 'X'=OEt, the optimum conditions necessary for the phase (C) to pass on to (B) are supplied by the ketonic group already present. The greater percentage of enol in β-diketones is evidently due to to the absence of any retarding group like OEt. In the case of symmetrical systems, *e.g.*, acetylacetone, dibenzoylmethane, triacetylmethane, the number of enol isomerides (*e.g.* phases A and B) remains fixed. But in the case of unsymmetrical systems *e.g.*, benzoylacetone, β-ketonic esters, the number of enol isomerides increases with the increase of carbonyl groups. The result of Meyer's method of bromination is the algebraic sum of the percentages of all the enol isomerides present in the tautomeric compounds. Hence, the bromination method is quite helpless in the estimation of any particular enol isomer. The iodometric method is capable of giving an exact measurement of one of the isomers (V) of thioketonic esters. The estimation of isomer (VI) could not be effected since no reagent could be available at present which would estimate it leaving the isomer (V) unaffected. The work is, however, in progress.

(V) (VI)

EXPERIMENTAL.

Thioketonic esters required in this investigation were prepared according to the method described by the author (*J. Indian Chem. Soc.*, 1938, **15**, 3).

In the case of ordinary mercaptans it was found that even at as low a temperature as $-7°$, iodine solution could be declourised as rapidly as at ordinary temperature with a sharp end-point. The incapability of ethyl crotonate to absorb any iodine indicated that the double bond present in enolised β-thioketonic esters could not share any iodine used for thiol estimation. On the other hand, the absence of any halogen in the resulting compound after titration confirmed that under the conditions of the experiment the thiocarbonyl group did not absorb iodine to form an additive compound. Error due to displacement of equilibrium in the course of titration was also found to be negligible since a quantity of the ester after treatment with iodine until the colour just persisted, on being kept at the room temperature (15°) for five minutes, required only a few drops of a dilute solution of iodine to bring the colour back. Considering the slowness of the rate of thiolisation it was thought desirable to add an excess of iodine to the solution of the ester and to titrate the residual iodine with dilute thiosulphate. The results from both the methods agreed well. The estimations were performed in all cases under identical conditions as far as possible.

A known quantity of the ester was taken in 10 c. c. of a solvent (ethyl alcohol) in a hard glass test-tube and kept at the required temperature for six hours. A titration flask containing alcoholic iodine (50 c.c.) of known strength and some ethyl alcohol in a separate flask, were cooled to $-7°$. The ester from the thermostat was quickly added to the cooled alcoholic iodine solution, and the remaining contents of the tube were washed in with cooled alcohol (10 c.c. at $-7°$). The mixture was allowed to stand for about 25 seconds and the excess of iodine was titrated against a standard thiosulphate solution. In an experiment with a mercaptan, it was found that iodine did not take more than 5 seconds to oxidise the thiol. All the thio-esters required in this investigation were kept at room temperature (15°) for 4 weeks before the thiol was estimated.

TABLE II.

Me. CS·CH_2·CO_2Et.

Ester.	Temp.	Iodine. (0·06722*N*)	Thiosulphate. (0·03126*N*)	Thiol.
0·4904 g.	30°	25 c.c. diluted with 25 c.c. EtOH	9·6 c.c.	41·08%
0·4900	60°	Do	12·2	38·7

TABLE II (*contd.*).

$Me{\cdot}CS{\cdot}CH{\cdot}(Me)CO_2Et$.

Ester.	Temp.	0·0505*N*-Iodine.	0·03239*N*-Thiosulphate	Thiol.
0·5276 g.	30°	50 c.c.	14·0 c.c.	62·87%
0·5517	40°	,,	12·0	61·97
0·5432	60°	,,	15·1	60·01

$Me{\cdot}CS{\cdot}CH{\cdot}(CH_2CH : Me_2)CO_2Et$.

Ester.	Temp.	0·0672*N*-Iodine.	0·03126*N*-Thiosulphate.	Thiol.
0·3750 g.	60°	30 c.c.	15·5 c.c.	64·42%

$Me{\cdot}CS{\cdot}CH{\cdot}CO_2Et$

Ester.	Temp.	0·06719*N*-Iodine.	0·02754*N*-Thiosulphate	Thiol.
0·5672 g.	30°	25 c.c., diluted with 25 c.c. of EtOH	3·2 c.c.	61·16%
0·5723	60°	Do	5·2	58·53

My thanks are due to Prof. Samuel Smiles, F.R.S., for his kind interest in this investigation and for the facilities granted.

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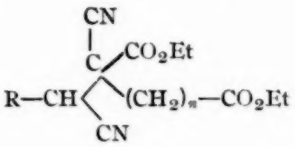
A NEW SYNTHETIC ROUTE TO POLYCYCLIC HYDRO-AROMATIC COMPOUNDS. SYNTHESIS OF 2:3-BENZ-*BICYCLO*-[0:3:3-]-Δ^2-OCTENE.

By Nripendra Nath Chatterjee.

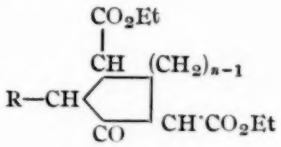
A general scheme for the synthesis of mono, bi, tri, and polynuclear hydroaromatic compounds has been described in the present communication. A synthesis of 2:3-benz-*bicyclo*-[0:3:3]-Δ^2-octene has been achieved by an application of the above method. Attempt has also been made to synthesise hexahydropentanthrylenemethane.

The object of this investigation is the exploitation of a new method of synthesis of polycyclic hydroaromatic compounds having œstrogenic and carcinogenic activity.

The method consists in condensing the sodium salt of the dicyano ester formed by the condensation of an aldehyde-cyanohydrin with ethyl sodiocyanoacetate with a monohalogenated ester to yield the product (A). This on hydrolysis gives an acid, the triethyl ester of which, when subjected to Dieckmanns, condensation, yields the product (B). The ester (B) on hydrolysis and decarboxylation yields a keto-acid, which serves as the starting material in building up other types of ring systems.



(A)



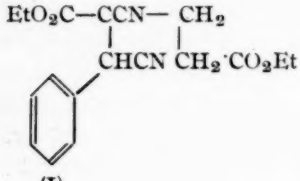
(B)

where R is either alkyl or other ring system and n may have values ranging from 1 to 3.

For the synthesis of 2:3-benz-*bicyclo*-[0:3:3]-Δ^2-octene (VII) the following method has been adopted. Benzaldehyde-cyanohydrin is allowed to react with the sodium salt of ethyl cyanoacetate when the sodium derivative of ethyl $\alpha\beta$-dicyano-β-phenylpropionate (A, R=Ph) is obtained. It is allowed to react with ethyl β-chloropropionate when diethyl-$\alpha\beta$-dicyano-α-phenyl-n-butane-$\beta\delta$-dicarboxylate (I) is obtained. When hydrolysed by means of sulphuric acid the latter yields an acid, the triethyl ester (II) of which undergoes cyclisation in presence of sodium in benzene to yield diethyl 2-phenyl*cyclo*pentanone-3:5-dicarboxylate (III).

This ester on hydrolysis and decarboxylation, yields 2-phenyl*cyclo*pentanone -3-carboxylic acid (IV). 2:3-Benz-*bicyclo*-[0:3:3]-Δ^2-octene-4-one (VI), obtained by the Clemmensen reduction and subsequent ring-closure of the keto-acid (IV) yields the required hydrocarbon (VII) on reduction.

For the synthesis of hexahydropentanthrylenemethane the ethyl ester of the keto-acid (IV) is condensed with ethyl bromoacetate by Reformatsky's reaction to give diethyl 2-phenyl*cyclo*pentanol-3-carboxylate-1-acetate (VIII). This is dehydrated to the corresponding unsaturated ester (IX) by Darzen's method and hydrogenated to diethyl 2-phenyl*cyclo*pentane 3-corboxylate-1-acetate (X). This ester on hydrolysis yields the corresponding dibasic acid which by internal Friedel-Craft's reaction, is converted into a substance which seems to be 8-ketohexahydropentanthryleneketone (XI).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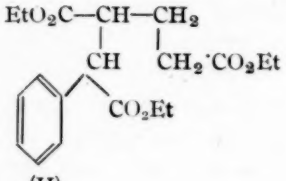
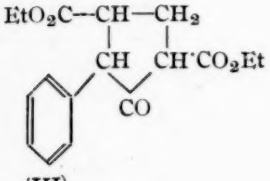

(II)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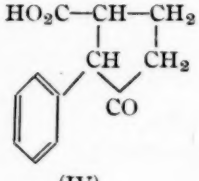
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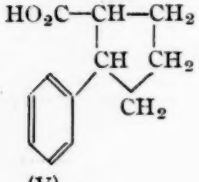
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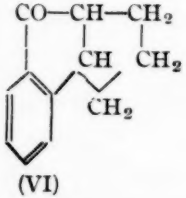
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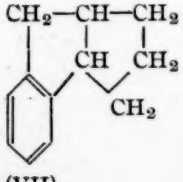
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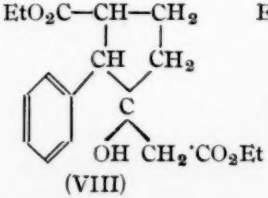
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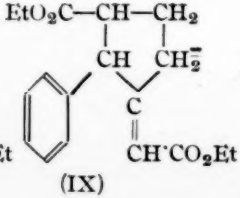
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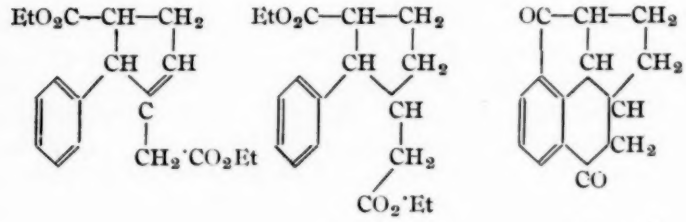
(IX)



(X) (XI)

Incidentally it might be mentioned that the above method is equally suitable for the synthesis of tricarboxylic acids with the carboxyl groups attached to three different carbon atoms, as well as for the synthesis of *cyclo*hexane and *cyclo*pentane derivatives.

Similar compounds have been obtained from benzaldehyde-cyanohydrin and ethyl γ-bromobutyrate and this will be the subject of the next communication. Investigations are in progress with other ring systems.

EXPERIMENTAL.

*Diethyl αβ-Dicyano-α-phenyl-*n*-butane-βδ-dicarboxylate* (I).—Sodium (11·6 g.) was dissolved in alcohol (140 c. c.) and ethyl cyanoacetate (56 g.) added. Mandelic nitrile (66 g.) was then added to the alcoholic solution containg the insoluble sodium compound in suspension, in small quantitites at a time. Considerable heat was generated after each addition, and the temperature was regulated by cooling. When all the nitrile had been added, the sodium compound passed into solution and the clear, brown liquid was allowed to stand for 12 hours for the completion of the reaction. The mixture was mixed with ethyl β-chloropropionate (64 g.) and after the initial reaction had abated was boiled under reflux for about 15 hours. The filtered liquid was diluted with water and extracted with ether; the ethereal solution was washed with water, dried and the ether removed. The dicyano ester distilled as a viscous liquid, b. p. 218-22°/5 mm., yield 85 g. (Found: C, 65·7; H, 6·4. $C_{18}H_{20}O_4N_2$ requires C, 65.8; H, 6·09 per cent).

The major portion of the liquid became solid on standing in a vacuum desiccator over sulphuric acid, oily impurities removed on porous plate and then recrystallised from ethyl alcohol. The ethyl ester was then obtained in colourless crystals, m. p. 81°. (Found: N, 9·0. $C_{18}H_{20}O_4N_2$ requiries N, 8·5 per cent).

*α-Phenyl-*n*-butane-αβδ-tricarboxylic Acid.*—The foregoing ester (20 c. c.) was mixed with sulphuric acid (70%, 120 c. c.) and boiled under reflux for 12 hours, the condenser being removed from time to time to allow

the alcohol formed to escape. The solution was then diluted with water, extracted with ether, and the ethereal solution was treated with sodium carbonate. On acidification of the carbonate solution the acid was obtained partly in the form of its acid-anhydride, which was heated on a water-bath with a solution of caustic alkali (15%) for 3-4 hours. The alkali solution was then acidified and extracted with ether. After remoing ether the product was kept in a desiccator where it solidified. It crystallised from dilute hydrochloric acid, m. p. 185° (decomp.), yield 10 g. (Found : C, 58·64 ; H, 5·29. $C_{13}H_{14}O_6$ requires C, 58·6 ; H, 5·2 per cent).

Triethyl α-Phenyl-n-butane-αβδ-tricarboxylate (II).—It was obiained in an almost quantitative yield from the acid by the alcohol vapour method. In a typical experiment the acid (30 g.), absolute alcohol (100 c. c.), concentrated sulphuric acid (9 c. c.), 3 litres of vapourised alcohol (3-4 hours) gave 35 g. of the ester, b. p. 187-94°/5 mm. (Found : C, 65·4 ; H, 7·3. $C_{19}H_{26}O_6$ requires C, 65·1 ; H, 7·4 per cent).

Diethyl 2-Phenylcyclopentanone-3 : 5-dicarboxylate (III).—A mixture of the foregoing ester (30 g.) and molecular sodium (4 g.) in dry benzene (75 c. c.) was refluxed for 10 minutes to start the reaction. The heating was discontinued until the vigour of the reaction abated and it was then continued for 2 hours. After cooling, the product was treated with cold dilute sulphuric acid and the benzene layer washed with aqueous sodium carbonate and with water, dried and evaporated. The residue in alcoholic solution gave a reddish violet colouration with ferric chloride. The ester was obtained as a pale yellow oil (18 g.), b. p. 184-92°/5 mm. (Found: C, 67·6 ; H, 6·5. $C_{17}H_{20}O_5$ requires C, 67·1 ; H, 6·5 per cent).

2-Phenylcyclopentanone-3-carboxylic Acid (IV).—The ester (III., 10 g.) was refluxed with excess of dilute sulphuric acid (20%) for 12 hours and the cooled solution saturated with ammonium sulphate and repeatedly extracted with ether, and the extract washed with water and dried. After removing the ether it was kept in a desiccator where it crystallised. Recrystallised from ether it melted at 114-15°. (Found : C, 70·8 ; H, 6·1. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·8 per cent).

2-Phenylcyclopentane-1-carboxylic Acid (V).—The pure keto-acid (IV, 5 g.), amalgamated zinc (40 g.), and concentrated hydrochloric acid (40 c.c.) were refluxed for 12 hours, more acid (40 c.c.) was then added and the heating continued for 12 hours. By extraction with ether a product was obtained which showed no properties of a ketone and boiled at 185-90°/10 mm. (Found : C, 75·6 ; H, 7·4. $C_{12}H_{14}O_2$ requires C, 75·7 ; H, 7·3 per cent).

2:3-*Benz*-bicyclo-[0:3:3]-Δ^2-*octene*-4-*one* (VI).—The foregoing acid (4 g.) was converted into its chloride by 1 hour's boiling with thionyl chloride (25 c.c.), excess of thionyl chloride being subsequently removed on the water-bath under reduced pressure. An ice-cold carbon disulphide solution (25 c.c.) of the chloride was treated with anhydrous aluminium chloride (3 g.) kept at 0° for 3 hours, the whole was heated to boiling and then decomposed with ice and hydrochloric acid. After working up in the usual manner the product was distilled at 127-32°/10 mm. (Found: C, 83·6; H, 6·8. $C_{13}H_{12}O$ requires C, 83·72; H, 6·9 per cent). The semicarbazone had m.p. 170°. (Found: N, 18·1. $C_{13}H_{15}ON_3$ requires N, 18·3 per cent).

2:3-*Benz*-bicyclo-[0:3:3]-Δ^2-*octene* (VII).—The foregoing ketone (2 g.) was reduced by boiling for 10 hours with concentrated hydrochloric acid (40 c.c.), water (40 c.c.) and amalgamated zinc (40 g.), concentrated hydrochloric acid (5 c.c.) being added after each hour. The hydrocarbon was separated with ether and distilled, b.p. 118°-24°/9 mm. (Found: C, 91·7; H, 8·8. $C_{12}H_{14}$ requires C, 91·1; H, 8·8 per cent).

Ethyl 2-*Phenyl*cyclo*pentanone*-3-*carboxylate*, prepared by refluxing a solution of the keto-acid (10 g.) in absolute alcohol (10 c.c.) saturated at 0° with hydrogen chloride, or by esterifying overnight with alcohol saturated with hydrogen chloride, formed a crystalline solid (10 g.). Recrystallised from alcohol, it melts at 60°. (Found: C, 72·3; H, 6·9. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·8 per cent). The semicarbazone crystallised from absolute alcohol, m.p. 173°. (Found: N, 14·68. $C_{15}H_{19}O_3N_3$ requires N, 14·53 per cent).

Diethyl 2-*Phenyl*cyclo*pentanol*-3-*carboxylate*-1-*acetate* (VIII).—A mixture of ethyl 3-phenyl*cyclo*pentanone-3-carboxylate (5 g.), ethyl bromoacetate (3·6 g.), dry zinc filings (purified by washing first with warm alkali and then with absolute alcohol and dry ether, 3 g.), dry and pure benzene (25 c.c.) was warmed on the water-bath until a reaction set in. The vigorous reaction caused the mixture to boil for 10 minutes without external heating. When this had subsided, the whole was boiled for 6 hours, and the product decomposed with ice-cold dilute sulphuric acid, washed, dried, and distilled. The hydroxy ester (5 g.) had b.p. 190-97°/6 mm. (Found: C, 67·26; H, 7·05. $C_{18}H_{24}O_5$ requires C, 67·5; H, 7·5 per cent).

Diethyl 2-*Phenyl*-Δ^1-cyclo*pentenylacetate*-3-*carboxylate* (IX).—Thionyl chloride (2·8 c.c.) was added slowly to a well-stirred ice-cold mixture of the foregoing hydroxy-ester (10 g.), anhydrous ether (30 c.c.) and pure pyridine (5 c.c.). Stirring and cooling were continued for 2 hours, the solution was then poured into ice-water, and ethereal solution was washed, dried, and distilled. Diethyl 2-phenyl-Δ^1-*cyclo*pentenylacetate-3-carboxylate formed a light

yellow liquid, b.p. 184°/5 mm. (Found : C, 71·61 ; H, 7·13. $C_{16}H_{22}O_4$ requires C, 71·52 ; H, 7·2 per cent).

Diethyl 2-Phenylcyclopentane-3-carboxylata-1-acetate (X).—The distilled unsaturated ester was hydrogenated in absolute alcoholic solution, platinum oxide catalyst being used. 6 G. of the substance in alcohol (50 c.c.) were shaken for 12 hours with platinum oxide (0·5 g.) in an atmosphere of hydrogen when the theoretical quantity of hydrogen was absorbed. Diethyl 2-phenyl*cyclo*pentane-3-carboxylate-1-acetate boiled at 175·77°/5mm. (Found : C, 70·4 ; H, 7·68. $C_{18}H_{24}O_4$ requires C, 71·0 ; H, 7·8 per cent).

2-Phenylcyclopentane-3-carboxylic-1-acetic Acid.—It was obtained by refluxing diethyl 2-phenyl*cyclo*pentane-3-carboxylate-1-acetate with alcoholic potash for 2 hours. The alcohol was distilled off and after dilution the unchanged ester was removed by means of ether. The aqueous layer was concentrated and after acidification the free acid was extracted with ether, the ether removed and the product kept in a vacuum desiccator when it solidified. It was finally crystallised from dilute acetic acid, m.p. 170-73°. (Found : C, 67·85; H, 6·39. $C_{14}H_{16}O_4$ requires C, 67·7 ; H, 6·4 per cent).

8-Ketohexahydropentanthryleneketone (XI).—The dibasic acid from the ester (X, 4 g.) was converted into its chloride by boiling with thionyl chloride (40 c.c.) for 2 hours, the excess of thionyl chloride being then removed on the water-bath under reduced pressure. A solution of the chloride in carbon disulphide at 0° was treated with anhydrous aluminium chloride (5 g.). After being kept in ice for 2 hours the whole was heated to boiling and then decomposed with ice and hydrochloric acid. After removing carbon disulphide it was taken up in ether. The ethereal solution was washed with sodium bicarbonate solution, dried and distilled. A small fraction (0·1 g.), probably (XI), obtained on distillation (the boiling point could not be recorded, as very small quantity of the substance was obtained) was analysed. (Found : C, 78·1; H, 5·9. $C_{14}H_{12}O_2$ requires C, 79·2; H, 5·6 per cent).

The author's respectful thanks are due to Sir P. C. Ray and Dr. P. C. Mitter for their kind interest in the work. The author is indebted to Mr. N. Ghosh for micro-analysis.

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THE PHENYLTHIOCARBAMIDES. A CONTRIBUTION TO THE STUDY OF THE TRAID -N·C·S-. PART VI. ACTION OF NITROUS ACID ON αβ-METHYL-PHENYLTHIOCARBAMIDE.

By Kunj Behari Lal and H. Krall.

The reaction has been studied to see the effect on the configuration of the thiocarbamide by (*i*) weak acid and (*ii*) strong acid. This is shown by the production of a nitroso derivative in (*i*) and an oxidation change in (*ii*). Under the conditions described half of the sulphur can be eliminated from the product of either change to result in the formation of a single base. $C_{16}H_{18}N_4S$.

The action of nitrous acid has been studied on phenyl-, and αα′-methylphenylthiocarbamides (Mehta and Krall, *J. Indian Chem. Soc.*, 1935, **12**, 635 ; Krall, *ibid.*, 1937, **14**, 478). In both cases in the presence of weak acid the production of nitrogen and accompanying end-products suggested their normal behaviour as amides. On the other hand, in the presence of strong acid the change was mainly one of oxidation resulting in the formation of Hector's base in the first case and a formamidine disulphide in the second.

$$2\,Ph{\cdot}NH{\cdot}CSNH_2 + 4HNO_2 = C_{14}H_{12}N_4S + S + 4NO + 2H_2O \quad \ldots (i)$$

$$2Ph(Me)N{\cdot}CSNH_2 + 2HNO_2 = [Ph(Me)NC(NH).\,S{-}]_2 + 2NO + H_2O \ldots (ii)$$

As an extension of this work the above reaction has been studied with αβ-methylphenylthiocarbamide.

When sodium nitrite reacts with the thiocarbamide in the presence of acetic acid, practically no gas is evolved and a nitroso derivative is formed thus:

$$Ph{\cdot}NH{\cdot}CS{\cdot}NHMe + HONO = Ph{\cdot}NH{\cdot}CSN(NO)Me + H_2O \quad \ldots (A)$$

The latter is decomposed by aqueous hydrochloric acid whereby nitric oxide is first evolved and then half of the sulphur is eliminated and the resulting solution yields a picrate from which the composition of the free base is deduced to be $C_{16}H_{18}N_4S$. The reaction, therefore, seems to be as follows:

$$2Ph{\cdot}NH{\cdot}CS{\cdot}N(NO)Me \longrightarrow \underset{\text{(a base)}}{C_{16}H_{18}N_4S} + 2NO + S \quad \ldots (B)$$

The free base, however, is not obtained pure as it decomposes in solution into phenylmustard oil and a resinous basic nitrogenous substance. With alkali the above nitroso derivative evolves nitrogen and phenylmustard oil is the main product. The fact that thiocarbanilide itself on treatment as in (A) does not react lends additional support for this mode of formation.

In the presence of hydrochloric acid, sodium nitrite reacts rapidly and produces initially an evanescent wine-red colour; nitric oxide (99%) is evolved and the solution gives a precipitate with aqueous picric acid. These facts indicate an oxidation change *e.g.*,

$$2Ph{\cdot}NH{\cdot}CS{\cdot}NHMe + 2HNO_2 = [Ph{\cdot}NH{\cdot}C(NMe)\,S{\cdot}]_2 + 2NO + H_2O \quad \ldots \text{(C)}$$

Varying amount of the nitroso derivative is always produced which is immediately decomposed in the acid reaction mixture. The base soon deposits half of the sulphur yielding another base which proves to be identical with the one obtained by decomposing the nitroso derivative with hydrochloric acid as in (B) above. The formation of nitroso derivative in the presence of a weak acid is an evidence for the existence of a thiocarbamide configuration, $Ph{\cdot}HN{\cdot}CS{\cdot}NHMe$. In the presence of strong acid, however, it is difficult to decide if the oxidation change referred to above is due to the conversion of the thiocarbamide into another tautomeride or else to the secondary decomposition of the nitroso derivative, because in either case the base obtained after elimination of sulphur happens to be identical.

In an attempt to synthesise diazothiols by oxidation of aromatic thiocarbamides with ferric chloride Chakervarti and De (*J. Indian Chem. Soc.*, 1928, **5**, 661) observed the formation of mustard oils and guanidine derivatives. It is, therefore, tempting to suggest that the base $C_{16}H_{18}N_4S$ may be an intermediate product during the formation of the expected diazothiol. This is being investigated.

EXPERIMENTAL.

Action of Nitrous Acid on αβ-Methylphenylthiocarbamide in presence of Acetic Acid.—The thiocarbamide (8·3 g.) was dissolved in 50% warm alcohol (200 c. c.) in a beaker and one equivalent of 99·2% sodium nitrite (3·475 g.) in water (50 c. c.) was added. When crystallisation had just started, the beaker was put in a water-bath and 2*N*-acetic acid (100 c.c.) was added in two or three instalments. A yellow precipitate was soon obtained with little evolution of nitric oxide. After 1 hour it was isolated in almost pure

form (yield 10 g.) and was recrystallised by the slow evaporation of its ethereal solution in golden yellow needles, m. p. 84° (decomp.). (Found: S, 15·7. $C_8H_9ON_3S$ requires S, 16·1 per cent). The substance decomposes on keeping. The aqueous mother liquors smelt of phenylmustard oil and later deposited some needles, m.p 137° (*vide infra*).

Decomposition of the Nitroso Derivative. (*a*) *With Acid.*—The nitroso derivative (4·9 g.) in alcohol (50 c.c) was warmed with *N*-hydrochloric acid (200 c c.) at 70-75° for 10 minutes. Nitric oxide was evolved and 0·39 g. of sulphur (half the theory, 0·4 g.) separated. The acid filtrate with alkali gave a base which could not be crystallised from the common solvents. In the dry state the precipitated base was fairly stable and melted at 82°. An alcoholic solution of the base (1·5 g.) and picric acid (1·2 g.) gave a picrate (cubes), m.p. 195°. [Found: N, 18·56 ; S, 6 $C_{16}H_{18}N_4S{\cdot}C_6H_2OH(NO_2)_3$ requires N, 18·6; S, 6·01 per cent]. On heating either alone or in solution the base decomposed into phenylmustard oil and a basic resinous substance. If an aqueous acidic solution of the base, as obtained after decomposing the nitroso derivative with hydrochloric acid, was treated with aqueous picric acid isomeric picrate was obtained. The latter crystallised from methyl alcohol (leaflets and needles) but decomposed on repeated crystallisation. [Found: N, 18·64; S, 6·1 per cent]. Its m.p. (153-54°) was slightly low due to the presence of traces of the isomer (cubes). Occasionally, under conditions not yet understood, the picrate, melting at 195°, was obtained even in this case.

(*b*) *With Alkali.*—The nitroso derivative (3·92 g.) was treated with cold *N*-sodium hydroxide (20 c. c.). Reaction began with the immediate evolution of nitogen and the alkaline reaction mixture contained an oil (2.5 c. c.) smelling of mustard oil and *iso*cyanide, and some sulphide. The separated oil after being washed with dilute hydrochloric acid gave phenylmustard oil (2.3 g ; theoretical 2·7 g.).

(*c*) *With Water.*—In aqueous alcoholic solution the nitroso derivative decomposed slowly evolving nitric oxide (58%), nitrogen (42%) and sulphur and phenylmustard oil were also formed. The latter seemed to disappear after some time and a nonbasic solid, m.p. 137°, containing S and N and stable to dilute alkali was also obtained.

Action of Sodium Nitrite on αβ-Methylphenylthiocarbamide in presence of Hydrochloric Acid (Gas Phase).—The thiocarbamide (0·083 g.) was introduced into a Lunge's nitrometer with the help of 1 c.c. of alcohol, 1 c. c. of water and the mixture treated with 1 c. c. of 4*N*-hydrochloric acid and one equivalent of *N*-sodium nitrite (0·5 c. c.). The reaction was brisk and began with the production of a red colour and some yellow nitroso derivative·

which decomposed after some time. Gas evolved (12·6 c. c. at 22° and 750 mm.) contained nitric oxide (99%). Excess of nitrous acid evolved more gas.

Action of Nitrous Acid on αβ-Methylphenylthiocarbamide in presence of Hydrochloric Acid (neglicting the gas phase).—The thiocarbamide (4·15 g.) was dissolved in 50% warm alcohol (100 c. c.) and 4*N*-hydrochloric acid (50 c. c.) was added. When crystallisation commenced, the solution was put in a water-bath and treated with an equivalent of sodium nitrite (1·737 g.) in water (50 c. c.). The reaction began with the production of red colouration and some nitroso derivative. The latter also went in solution after some time. The clear solution was examined as follows:—

(*i*) 50 C. c. of the solution were neutralised with ammonia when an oil and later a solid appeared. The latter yielded sulphur partly on teatment with 4*N*-hydrochloric acid and partly from the acid solution so obtained.

(*ii*) 0·5 C.c. of the solution was treated with aqueous picric acid when a yellow precipitate (2 g.) was obtained. On crystallisation from methyl alcohol, however, sulphur separated and a picrate (needles and leaflets), m.p. 158° crystallised out. [Found: N, 18·65 ; S, 6·15. $C_{16}H_{18}N_4S{\cdot}C_6H_2OH(NO_2)_3$ requires N, 18·61 ; S, 6·01 per cent]. Mixed m.p. with the similar picrate obtained previously [*cf.* (*a*) p. 219] 155-56°.

(*iii*) 50 C. c. of the solution on standing (1 hour) or on warming at 80° deposited 0·086 g. of sulphur (theoretical 0·1 g.) and the resulting solution gave with aqueous picric acid the picrate, m.p. 153-54° identical with that in (*ii*).

(*iv*) 50 C. c. of the solution were warmed at 80° and filtered from separated sulphur (0·09 g.). The acid filtrate with alkali gave a base which in alcoholic solution gave the picrate (cubes), m. p. 195° with picric acid as in (*a*).

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THE PHENYLTHIOCARBAMIDES. A CONTRIBUTION TO THE STUDY OF THE TRIAD -N·C·S-. PART VII. A RE-INVESTIGATION OF THE EXTENT ON SOME HYDROLYTIC DECOMPOSITIONS OF PHENYLTHIOCARBAMIDE. REACTIONS OF SODIUM ETHOXIDE ON PHENYLTHIOCARBAMIDE AND OF ACETIC ANHYDRIDE AND HYDROLYTIC AGENTS ON $a\alpha'$- AND $a\beta$- METHYLPHENYLTHIOCARBAMIDES.

By Kunj Behari Lal and H. Krall.

In continuation of previous hydrolytic studies upon phenylthiocarbamide (I), some of which needed reinvestigation, it became desirable to extend the hydrolytic reactions to the *unsym*- (II) and *sym*. methyl (III) homologues of (I) and secondly try the action of (*a*) a strong alkali (NaOEt) on (I) and, (*b*) a strong acidic reagent (Ac_2O) on (II) and (III) respectively. (*a*) in presence of alcohol yields hydrogen sulphide and phenylcyanamide but the reaction is otherwise in its absence. This, together with reactions of (II) show the importance of the hydrogen atom of the anilino group in this change. (III) resembles (I) in its reactions but (II) differs from either of these in the case of its decomposition with various reagents. Results recorded for (I)-(II) indicate that increasing acidity does not effect the hydrolytic de.omposition in the manner supposed previously.

Hydrolytic agents decompose phenylthiocarbamide (Mehta and Krall, *J. Indian Chem. Soc.*, 1935, **12**, 635) into hydrogen sulphide and phenylcyanamide, aniline and thiocyanic acid, ammonia and phenylmustard oil, thus :

$$Ph{\cdot}NH{\cdot}CS{\cdot}NH_2 \longrightarrow H_2S + Ph{\cdot}NH{\cdot}CN \quad \ldots \quad (i)$$

$$Ph{\cdot}NH{\cdot}CS{\cdot}NH_2 \longrightarrow PhNH_2 + HSCN \quad \ldots \quad (ii)$$

$$Ph{\cdot}NH{\cdot}CS{\cdot}NH_2 \longrightarrow NH_3 + Ph{\cdot}NCS \quad \ldots \quad (iii)$$

and the extent of each of these changes was found to depend upon the acidity of the medium. Later, however, evidence accumulated to show that analytical difficulties had led to some erroneous conclusions with regard to changes (*ii*) and (*iii*) which are now reinvestigated. Further, the behaviour of phenylthiocarbamide towards sodium ethoxide and of $a\alpha'$- and $a\beta$-methylphenylthiocarbamides towards acidic reagents, water and alkali have been studied for comparison. To estimate small amount of thiocyanic acid formed as in (*ii*) in presence of phenylthiocarbamide two methods

have now been devised, one of which depends on the ready formation of a nickel tetrapyridine thiocyanate complex $[NiPy_4(CNS)_2]$ (Spacu, *Bull. Soc. Stiinte Cluj*, 1922, **1**, 314 ; 1923, **2**, 585) which also has the advantage of being applicable in presence of chlorides, and the other on the removal of phenylthiocarbamide from solution by ammoniacal silver nitrate (*vide* experimental) which can also be utilised to estimate small amount of mustard oil produced in change (*iii*).

The results obtained by these methods show that change (*ii*) is 2-5 % in one hour and increasing acidity does not materially increase the change. Also, change (*iii*) can occur to a small extent in aqueous solution as well as in presence of strong acids.

When alcoholic solutions of phenylthiocarbamide and sodium ethoxide are mixed and boiled, considerable sulphide is produced according to change (*i*). In the absence of alcohol, however, if the residue, supposed to contain the sodium salt of the thiocarbamide, be carefully heated, a residue of sodium sulphide is not produced appreciably, sodium thiocyanate predominating, and complex decompositions like those obtained by dry-heating phenylthiocarbamide alone (Davis and Underwood, *J. Amer. Chem. Soc.*, 1922, **44**, 2802) occur.

$\alpha\alpha'$-Methylphenylthiocarbamide exhibits a striking contrast with the behaviour of either the parent phenylthiocarbamide or the *sym.* isomer in the ease with which it is decomposed by the various reagents. Thus when boiled with acetic anhydride, *N*-hydrochloric acid, water or *N*-sodium hydroxide it decomposes mainly into methylaniline and thiocyanic acid

$$Ph(Me)\cdot NCS\cdot NH_2 \longrightarrow Ph(Me)NH + HCNS$$

which occurs on mere dissolution in the first reagent. With the aqueous acid the change in one hour takes place twice as rapidly (37%) as with alkali (17%) and in both cases is greater than in water (9 %). Little hydrogen sulphide is produced in alkaline solutions.

Acetic anhydride, hydrochloric acid, and water decompose $\alpha\beta$-methylphenylthiocarbamide into phenylmustard oil and only traces of methyl mustard oil :

$$Ph\cdot HN\cdot CS\cdot NHMe \begin{cases} \longrightarrow MeNH_2 + PhNCS \\ \longrightarrow PhNH_2 + MeNCS \end{cases}$$

The change with acetic anhydride is maximum after six minutes (70%) and appears to take place through the initial formation of the acetyl compound of the thiocarbamide (*cf.* Werner, *J. Chem. Soc.*, 1891, **59**, 396). With

the aqueous acid, however, it is slow (5·4% in 1 hour) and more so with water. When boiled with alkali, sulphide is produced (12%) and from analogy with the parent compound it is expected to be formed thus :

$$Ph{\cdot}NH{\cdot}CS{\cdot}NHMe \longrightarrow H_2S + Ph{\cdot}NC{\cdot}NMe$$

Traces of aniline, methylamine, polysulphides have been found but we have not identified the above cyanamide nor the oxygen urea from it which is expected to be the product of such reactions.

These experiments show a formal similarity in the behaviour of $\alpha\beta$-methylphenylthiocarbamide and phenylthiocarbamide. In the latter case the slight diminution of change (*ii*) in relatively concentrated acid or in water is analogous to that observed by Fawsitt with methyl-, and $\alpha\alpha'$-dimethyl carbamides (*J. Chem. Soc.*, 1904, **85**, 1584, ; 1905, **87**, 474) but the explanations advanced by him to account for such results do not generally fit in our case with the *unsym.*-methyl homologue. The possibility of salt formation to some extent in acid solution cannot, however, be overlooked and it is likely that the cation so formed, with its electromeric change (*cf.* Lecher, *Annalen*, 1925, **445**, 35) determines the extent of such decompositions as (*ii*) and (*iii*).

$$\left[PhHN{\cdot}\overset{\overset{\oplus}{NH_2}}{\underset{SH}{C}}{=}NH \rightleftharpoons PhHN{=}\overset{\overset{\oplus}{NH_2}}{\underset{SH}{C}}{-}NH \right] \overset{\ominus}{Cl}$$

Comparing the reactions of sodium ethoxide in the presence of alcohol and on dry heating with phenylthiocarbamide it appears that it is the hydrogen of the anilino group of the thiocarbamide and for that matter of the *sym.* compound, which is concerned in the formation of hydrogen sulphide and a cyanamide derivative, the functioning of this hydrogen atom being suppressed in the absence of a solvent. This view is amply supported by the behaviour of $\alpha\alpha'$-methylphenylthiocarbamide under like conditions.

In all acidic and aqueous hydrolytic experiments a small amount of carbon oxysulphide is invariably formed which is not accounted for by the decomposition of thiocyanic acid or of mustard oil under the conditions of experiment. This fact taken in conjunction with the reaction of acetic anhydride throws a doubt on the view that thiocyanic acid or mustard oil is produced by the spontaneous dissociation of the thiocarbamide in solution into neutral molecules. Evidently there is some chemical combination with the solvent. Fearon (*Biochem. J.*, 1936, **30**, 1652) has shown that

cyanic acid is only a dehydration product of carbamic acid during the zymolysis of urea.

EXPERIMENTAL.

Hydrolytic Experiments with Phenylthiocarbamide : Procedure and Methods of Analysis.

Estimation of Thiocyanic Acid. (*a*).—To precipitate out nickel tetrapyridine thiocyanate [$NiPy_4$ $(CNS)_2$] (Spacu, *loc. cit.*), the slightly acid aqueous solution containing the thiocyanate (up to 0. 1 g. CNS' in 150 c.c.) was treated in a 250 c. c. flask with pyridine (7·5 c.c.) and a known excess of 0·1 *N*-nickel sulphate (10-20 c. c.) till the supernatant liquid acquired after the settling of the precipitate a bluish tinge. The liquid was then made upto 250 c. c. with water, mixed, and filtered through a dry filter into a separating funnel. Traces of the complex were removed from solution by extracting thrice with chloroform (5 c. c.) which did not affect excess of nickel present otherwise. For determining the latter, 200 c. c. of the solution were treated with moderate excess of 2 *N*-sodium hydroxide which precipitated nickelous hydroxide quantitatively from aqueous pyridine solution. Phenylthiocarbamide and moderate amount of aniline did not interfere but diphenylguanidine did. The precipitate was washed free of phenylthiocarbamide and chloride by hot dilute solution of potassium nitrate, redissolved into a 250 c.c. flask by warm dilute nitric acid and the solution made barely acid. Nickel was estimated in the latter solution by again precipitating the thiocyanate complex (*cf.* Dobbins and Sanders, *Ing. Eng. Chem. Anal. Ed.*, 1934, **6**, 459) with 0·1 *N*-potassium thiocyanate, pyridine (3 c.c.) and excess of potassium thiocyanate in an aliquote of 100 c.c. liquid titrated back with silver nitrate.

(*b*) The thiocarbamide present in slightly acid aqueous solution containing upto 0·1 g. of CNS' in 100-150 c. c. solution was desulphurised with *N*-silver nitrate (3-4 c. c.) in presence of 8% ammonia (60 c. c.) and the mixture heated at 80° for 1 hour. On cooling, excess of silver was removed by passing hydrogen sulphide for a few seconds through the ammoniacal solution. The latter was immediately freed of hydrogen sulphide by addition of slight excess of cadmium sulphate, filtered from insoluble matter and neutralised with nitric acid, adding 2 c. c. in excess. The thiocyanic acid was then estimated by adding excess of 0·1 *N*-silver nitrate and titrating back with 0·1 *N*-potassium thiocyanate. The results by these two methods agreed closely and were accurate.

Estimation of Phenylmustard Oil.—The thiocarbamide was steam dis-

tilled in the presence of the hydrolytic agent and the aqueous distillate (150-200 c. c. for 1 hr.) treated with *N*-silver nitrate (3-5 c. c.), 8% ammonia (60 c. c.) and heated under reflux at 80° for 2 hours. The precipitated silver sulphide was weighed on a tared filter paper and the amount of mustard oil calculated.

Detection and Determination of Carbon Oxysulphide.—The upper end of the reflux condenser was connected with 5 absorption tubes containing in order strongly acidified copper sulphate solution (10 c. c.), 33% aqueous caustic potash solution (8 c. c.), pyridine (5 c. c.) and finally alcoholic caustic potash (1 g. KOH + 2. c c. water + 2 c. c. alcohol ; 15 c. c. and 10 c. c.). At the end of boiling, the air in the apparatus was driven through the above reagents by aspiration. The alcoholic solutions were mixed and concentrated, oxidised with bromine water and the sulphate so formed was estimated. Ammonium thiocyanate solution when boiled with excess of *N*-hydrochloric acid gave no sulphate, whilst with phenylmustard oil it was negligible.

Estimation of Ammonia in Aqueous Hydrolytic Liquors.—This depended on the observation of Foreman (*Biochem. J.*, 1920, **14**, 451; Gortner, "Outlines of Biochemistry", 1929, p. 328) that in 85% alcohol the salts of ammonia and amines with strong acids behave as free acids. Two samples (20 g.) were taken. In one free acid was determined by titration with alkali, and in the other absolute alcohol (180 c. c.) was added to make a 85% solution. The latter was titrated with alkali (*N*/40) using phenolphthalein as indicator. The difference gave the amount of ammonia in solution.

Table I records the results obtained by above methods on treating phenylcarbamide (5 g.) with an equivalent of the hydrolytic agent in concentrations stated therein. The figures (mols./100 mols. thiocarbamide) for carbon oxysulphide are for 2 hours' and the rest for 1 hour's boiling.

TABLE I.

	Dilution of carbamide and hydrolytic agent.	Hydrolytic agents		
		HCl.	Water.	NaOH.
Thiocyanic acid	1·00*N*	2·5	1·9	3·1
	0·66	4·0	2·6	...
	0·25	5·4	3·8	...
Phenylmustard oil	0·66	1·4	...	
	0·25	...	1·2	
Carbon oxysulphide	0·66	3·1	...	
	0·25	...	1·4	

In *N*-aqueous and acidic solutions some of the substance remained undisssolved. In another experiment a 0·25 *N*-aqueous solution of phenylthiocarbamide was boiled for 2 hours and ammonia and carbon oxysulphide also estimated (Found: HCNS, 6·7; PhNCS, 3·0; COS, 1·4; NH_3, 5%). During boiling, thiocarbanilide (3%) often got deposited on the inside of the reflux condenser tubing. Thus, the occurrence of decomposition of the thiocarbamide into ammonia and phenylmustard oil even in aqueous solutions was clearly demonstrated.

Phenylthiocarbamide and Sodium Ethoxide.—Phenylthiocarbamide (7·6 g.) in absolute alcohol (20 c. c.) was boiled with sodium (1·15 g.) in alcohol (30 c. c.) for 2 hours. No ammoniacal or any other odour came and the alcoholic solution contained sulphide and traces of thiocyanic acid. (Found: H_2S, 15; HCNS, 0·2%). In another experiment the alcohol was evaporated off and the residue dry-heated. The distillate contained traces of ammonia, aniline and thiocarbanilide and the residue contained sodium thiocyanate and traces of sulphide. On heating at a lower temperature (135-45°) for 2 hours, few drops of oil appeared in the distillate and a little subiimate was formed (ammonium sulphide). The residue contained thiocarbanilide, aniline, sulphide and sodium thiocyanate. (Found: H_2S, 12; HCNS, 64%). The last was the main product (Found: CNS′, 71·2. NaCNS requires CNS′, 71·6 per cent) that crystallised out on concentrating an alcoholic extract of the residue.

Hydrolytic Experiments with the Methylphenylthiocarbamides.

aa′-Methylphenylthiocarbamide and Acetic Anhydride.—The thiocarbamide (8·3 g.) and acetic anhydride (10 g.) were boiled under reflux for 3·15 minutes. The reddish solution contained methylaniline and thiocyanic acid which decomposed on longer heating. The acetyl compound of Doran and Dixon (*J. Chem. Soc.*, 1905, **87**, 339) was not identified in this experiment nor could it be prepared by Hugershoff's method (*Ber.*, 1899, **32**, 3649).

aa′-Methylphenylthiocarbamide and N-Hydrochloric Acid, Water and N-Sodium Hydroxide.—The thiocarbamide (8·3 g.) was refluxed with each of the above hydrolytic agents (50 c. c.) for 1 hour. The reaction mixture, which smelt of methylaniline in the last two cases, was cooled and analysed for thiocyanic acid (Volhard's method) and the results were checked by colourimetric measurements and estimating residual alkalinity respectively. Traces of hydrogen sulphide formed in the last case were also estimated.

TABLE II.

Hydrolytic agent.	Thiocyanic acid.	Hydrogen sulphide.
Hydrochloric acid	37	Traces.
Water	8·5	Nil
Sodium hydroxide	17·0	1·2

These changes were halved in $N/4$ solutions. In a similar experiment with N-alkali the boiling was done for 4 hours. (Found HCNS, 50; H_2S, 7%).

αβ-Methylphenylthiocarbamide and Acetic Anhydride.—The thiocarbamide (8·3 g.) and acetic anhydride (10 g.) were boiled for 3-15 minutes. The mustard oil formed was steam distilled and weighed.

TABLE III.

Time (min.)	...	3	6	10	15
Mustard oil (g.)	...	3·3	4·4	3·35	1·15

The yield of the oil diminished after six minutes due to the secondary change namely

$$PhNCS + AcOH = PhNHAc + COS$$

and acetanilide was present in the residual mixture after 4-5 minutes. Below 3 minutes a solid (m. p. 82°) could be isolated and was found to be identical with the acetyl derivative of the thiocarbamide prepared by Hugershoff's method (*loc. cit.*), m. p. and mixed m.p. 82°.

In the above experiments the mother liquors smelt of methylamine on warming with alkali. Also, on distilling together the four samples of mustard oil few drops of oil of b. p. 116-125° were obtained and were shown to contain methylmustard oil after conversion into αβ-methylphenylthiocarbamide. The latter formed an unstable yellow nitroso derivative (separate communication) with aqueous sodium nitrite and acetic acid. Thiocarbanilide apparently did not react under these conditions.

αβ-Methylphenylthiocarbamide and Hydrochloric Acid and Water.—The thiocarbamide (5·53 g.) and N-Hydrochloric acid (33 c. c.) diluted to 50 c. c. were steam distilled for 1 hour and the distillate treated with ammoniacal silver nitrate as in the case of the parent compound (Found: Ag_2S, 5·4%). In another experiment the distillate was extracted with chloroform and the oil converted into thiocarbamides. Mostly thiocarbanilide

was formed whilst small amount of αβ-methylphenylthiocarbamide was also shown to be present as in the case of acetic anhydride. The residual mixture smelt of methylamine on basification and no emulsion of aniline was visible.

With water (50 c. c.) there was hardly any change but on dilution (132 c.c.) few drops of mustard oils came over in the steam distillate (Found: Ag_2S, 2%).

αβ-Methylphenylthiocarbamide and N-Sodium Hydroxide.—The thiocarbamide (5·53 g.) and alkali (33 c. c.) were refluxed together for 1 hour. Traces of methylamine and aniline were produced, whilst the alkaline reaction mixture contained mainly unchanged thiocarbamide and sulphide (Found: H_2S, 12%). The estimation of the latter by titration with alkaline zinc solution presented difficulty because nickel sulphate which was used as an external indicator gave a persistent yellow colouration at the end-point. This was also verified on a large scale and showed the presence of traces of polysulphides (*cf.* Mermet, *Bull. Soc. chim.*, 1875, *ii*, **24**, 433). To get a control over the above figure, the precipitated zinc sulphide was filtered, washed and estimated iodometrically (Found: H_2S, 17%). On prolonged boiling (4 hours) the alkaline solution contained more sulphide (35%), unchanged thiocarbamide and polysulphides (traces) and smelt appreciably of aniline and methylamine, but no other product was found.

With αα-and αβ-methylphenylthiocarbamides carbon oxysulphide was detected as in the case of the parent compound but was not estimated.

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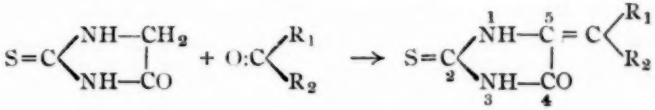
DYES DERIVED FROM THIOHYDANTOIN. PART III.

By G. P. Pendse.

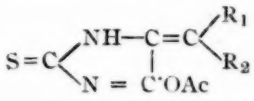
In continuation of the previous work thiohydantoin has been condensed with aliphatic and aromatic ketones and quinones with acetic anhydride as condensing agent. The condensation products have been isolated in keto form.

In previous communications thiohydantoin has been condensed with a number of nitroso and *iso*nitroso compounds (Pendse and Dutt, *J. Indian Chem. Soc.*, 1930, **7**, 953), and with aromatic aldehydes (Namjoshi and Dutt, *ibid.*, 1931, **8**, 241 ; Ghosh, *ibid.*, 1934, **11**, 27). To complete this series of investigations thiohydantoin has been condensed with aliphatic and aromatic ketones and quinones using acetic anhydride as the condensing agent.



(I)

With acetic anhydride unstable acetyl derivatives, presumably of the type (II) are generally formed, which are, however, very easily hydrolysed by dilute alkalis (or by boiling water) to the free keto types (I).



(II)

On account of the difficulty of purification of the acetyl derivatives due to their unstable nature, the condensation products described in this paper have been obtained in the free keto-form (I). As is expected the condensation products are less coloured than those obtained with aldehydes and much less than with nitroso and *iso*nitroso compounds. Where there are more than one keto groups in the compound, it has been observed that only one molecule of the ketone or quinone condenses with one molecule of thiohydantoin.

The following ketones have been condensed: with thiohydantoin. (1) phenanthraquinone, (2) tetramethyldiamidobenzophenone (3) acenaphthoquinone, (4) isatin, (5) fluorenone, (6) alizarin, (7) benzil, (8) dibenzylideneacetone, (9) *p*-benzoquinone and (10) anthraquinone.

Unsuccessful attempts were also made to condense the following:—

(1) Acetone, (2) methylpropylketone, (3) diethylketone, (4) *cyclo*-pentanone, (6) coumarin, (7) acetophenone.

Most of the condensation products are crystalline substances, and dye beautiful light shades on wool and silk.

EXPERIMENTAL.

5-Phenanthraquinonethiohydantoin.—A mixture of thiohydantoin (1·16 g.), phenanthraquinone (2 g.) and acetic anhydride (about 15 c.c.) was heated under reflux for about 1 hour. The reaction which was very vigorous at first gradually subsided and it was complete when the mixture assumed a brownish yellow shade. The mixture was cooled and then poured into water when a brown precipitate came down. This was filtered off, washed with hot water and crystallised from alcohol.

TABLE I.

(Thiohydantoin = T.

Name.	Appearance.	M.p.	Wave-length of absorption shadow.	Analysis. Found.	Required
5-Phenanthraquinone-T	Greenish brown needles	146°	4513Å	S, 10·23%	10·46
5-Tetramethyldiamino-benzophenone-T	Light yellow needles	166°	4450	9·16	8·75
5-Acenaphthoquinone-T	Chocolate brown powder	Above. 260°	4695	11·20	11·43
5-Isatin-T	Reddish brown prisms	„	4764	13·36	13·73
5-Fluorenone-T	Greyish yellow needles	102°	4410	11·72	11·41
(1 :2-Dihydroxyanthra-quinone-T 5-Alizarin-T	Yellowish green prisms	157°	5600	9·79	9·47
5-Benzil-T or	Greenish yellow flakes	93°	4630	10·64	10·39
5-Dibenzylideneacetone-T	Light brown flakes	113°	4710	9·23	9·58
5-*p*-Benzoquinone-T	Orange-brown needles	136°	4875	15·64	15·83
5-Anthraquinone-T	Light yellow flakes	167°	4890	10·37	10·46

Condensations of thiohydantoin with other ketones were similarly carried out with very slight modifications to suit individual cases. In general the condensation products are fairly soluble in ethyl and methyl alcohol, ethyl acetate, chloroform, and sparingly soluble in benzene, ether and carbon tetrachloride. Mineral acids dissolve the substances giving yellow to orange coloured solutions from which the original substances can be precipitated by addition of water. When to the alcoholic solutions of these dyes a little caustic soda is added the colour in many cases is considerably intensified. In several cases the substances can be precipitated by addition of dilute acids from dilute solution in alkali. The compounds are described in Table I.

The author takes the opportunity of expressing his indebtedness to Dr. S. Dutt of the Allahabad University for his valuable guidance and the interest he took in this work and to the authorities of the Victoria College, Gwalior, for the facilities given to him during the progress of this work.

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REVIEW.

Clowes and Coleman's Quantitative Chemical Analysis. EDITED AND REVISED BY D. STOCKDALE AND J. DEXTER, FOURTEENTH EDITION. PUBLISHED BY J. & A. CHURCHILL, LTD., LONDON, 1938. PP. 617 ; PRICE 18 SH.

This is the fourteenth and revised edition of Clowes and Coleman's Quantitative Chemical Analysis which the students of chemistry have long been quite familiar. That the publication with necessary revision of the fourteenth edition of a book, written nearly a half a century back, has been found necessary, speaks by itself of its popularity and usefulness. This new edition has been considerably enlarged by the inclusion of many modern methods of analysis, both inorganic and organic. A section on electrometric, electrolytic and colorimetric analysis has also been added. In all other respects, the book practically retains the main features of the previous edition. There can be no doubt that the present volume will justly maintain, if not enhance, the reputation and usefulness of its predecessors.

The book can be confidently recommended as a laboratory companion to the senior students of both pure and technical chemistry.

P. R.

Annual Review of Biochemical and Allied Research in India. Vol. VIII. PUBLISHED BY THE SOCIETY OF BIOLOGICAL CHEMISTS, INDIA, BANGALORE. PRICE RS. 3 OR 6 SH.

This is the eighth volume of the review published by the Society of Biological Chemists, India. It deals briefly but comprehensively with the contributions that all workers in India and Indian research workers abroad have made to the advancement of biochemical research in all its aspects during the last year. The publication is divided into the following chapters, written by different authors : Proteins, Enzymes, Pharmacology, Human Physiology, Human Pathology and Bacteriology, Foods and Nutrition, Microbiology and Fermentation, Plant Physiology, Chemistry of Plant Products, Phytopathology-Mycology, Phytopathology-Entomology, Soils, Fertilizers and Manures, Animal Nutrition and Dairy Science, Veterinary Science.

The review has fully justified the reason for its existence, viz., to present to workers in India and abroad with a bird's eye picture of the entire field of biochemical and allied research in India. The importance of such a presentation becomes apparent when it is remembered that the researches are published in a bewildering variety of journals and the reveiw, therefore, enables the reader to have a stock-taking of the progress of biochemistry in India. It brings into relief both the more important contributions made by Indians and also the gaps in the general front of biochemical advance, which require to be filled up. The Review is very readable and is remarkably free from errors. It is wellprinted and may be warmly recommended to all concerned.

B. G.

THE EFFECT OF HYDROGEN ION CONCENTRATION, ELECTROLYTES, NORMAL AND IMMUNE SERA ON THE CATAPHORETIC VELOCITY OF *LEISHMANIA TROPICA*. PART I.

By B. N. Ghosh and S. S. De.

The cataphoretic velocities of bacterial suspension in presence of electrolytes and non-electrolytes, normal and immune sera have been investigated.

The electrical charge of *Leishmania tropica* in a solution of sodium chloride of p_H 7·0 is negative and the cataphoretic velocity increases as the concentration of sodium chloride decreases. The nature of the electrical charge on *Leishmania tropica* is reversed between p_H 3·0 and p_H 3·4. Below p_H 3·0, they are positively charged and above p_H 3·4 they are negatively charged.

Both immune and normal sera, obtained from rabbits, decrease the cataphoretic velocity of *Leishmania tropica*. The effect is, however, much more pronounced with immune serum than with normal serum. The iso-electric point of *Leishmania tropica*, sensitised with immune serum, is in the neighbourhood of p_H 5·3.

The cataphoretic velocity of bacterial suspensions in presence of electrolytes, non-electrolytes, normal and immune sera has been extensively investigated and as a result of such investigation much valuable information on the relation of electrokinetic potential to agglutination (Northrop and de Kriuf, *J. Gen. Physiol.*, 1921, **4**, 639, 655), phagocytability (Topley, "Outline of Immunity", 1933, p. 77), toxigencity, especially of the diphtheria group (Falk *et al*, *J. Bacteriol.*, 1928, **15**, 6; Thompson, *Amer. J. Hyg.*, 1932, **15**, 712) and transition from the 'smooth' to 'rough' variety (Mudd and Mudd, *J. Exp. Med.*, 1927, **45**, 173) has been obtained. There are, however, very few such measurements in the case of protozoal bodies An investigation was, therefore, undertaken to ascertain the effect of electrolytes, hydrogen ion concentration and of normal and immune sera on the cataphoretic velocity of *Leishmania tropica* in aqueous suspension. The results so far obtained are recorded in this paper.

Experimental.

The micro-cataphoretic method was used in these experiments. The cell was a flat rectangular one, of the type used by Freundlich and Abramson (*Z. physikal. Chem.*, 1927, **128**, 25) with this slight modification that it was provided with two detachable side-tubes, one on each side. The joints were carefully ground so as to be leak-proof. Each side-tube carried

an electrode vessel and a three-way stop-cock. The cell was cleansed thoroughly before use and then filled with the desired suspension of *Leishmania tropica*, care being taken to remove all air bubbles. The electrode vessels were filled with copper sulphate solution into which dipped copper electrodes. The connection between the cell and the electrode vessels was established through agar bridges. Just before taking the actual reading, the electrodes were connected with the 220 volt lighting circuit. The current flowing through the system was indicated by a sensitive milliammeter included in the circuit. The velocity of the protozoal bodies was measured at two depths $d/6$ and $d/2$ inside the cell, where 'd' stands for the depth of the cell. For the cell used by us d was found to be 0·81 mm. The actual velocity of the protozoal bodies was calculated from the formula,

$$V = (\tfrac{3}{4}V_1 + \tfrac{1}{4}V_2)$$

where V_1 and V_2 are the observed velocities at depths $d/6$ and $d/2$ respectively inside the cell, as already mentioned.

Effect of Sodium Chloride on the Cataphoretic Velocity.

The cataphoretic velocity of *Leishmania tropica* in solutions of different concentrations of sodium chloride having p_H 7·0 was measured and the results are recorded in Table I. The velocity is expressed in cm./sec. per volt/cm. The micro-organisms are found to be negatively charged in these solutions. It will also be noticed from the data given in Table I that the cataphoretic velocity of the protozoa increases as the concentration of sodium chloride diminishes.

TABLE I.

Charge of protozoa—negative.

Percentage conc. of NaCl.	*V.	Percentage conc. of NaCl.	*V.
1·0	18·4	0·4	29·4
0·9	18·9	0·3	34·0
0·8	19·3	0·2	40·1
0·7	20·6	0·1	55·2
0·6	22·8	0·05	59·4
0·5	25·6	0·02	59·9

* V here and in the following tables indicates the cataphoretic velocities in cm. per sec. per volt/cm. multiplied by 10^5.

Effect of Hydrogen Ion Concentration on the Cataphoretic Velocity.

The effect of hydrogen ion concentration on the cataphoretic velocity of *Leishmania tropica*, suspended in aqueous solutions of glucose and sodium chloride, was determined and the results are recorded in Table II. Citrate-phosphate buffers were used for the p_H range 2·2 to 8·0 and above that range borate buffers were used. The + and − signs used before the figures in columns 2 and 3 indicate the nature of the electrical charge carried by the micro-organisms. It will be noticed from the data recorded in Table II that the electrical charge on the surface of the protozoal bodies is reversed between p_H 3·0 and p_H 3·4. At or below p_H 3·0, the electrical charge of the suspension is positive and its magnitude increases as the p_H decreases. Again at or above p_H 3·4, the charge is negative and its magnitude increases as the p_H increases within the range investigated.

TABLE II.

p_H.	Glucose soln. (V).	0·2% NaCl soln. (V).	p_H.	Glucose soln. (V).	0·2% NaCl soln. (V).
2·2	+23·7	+19·6	5·0	−43·4	−35·7
2·5	+20·8	+18·4	6·0	−53·5	−43·1
2·8	+18·0	+15·9	7·0	−61·4	−48·3
3·0	+ 8·9	+ 8·0	8·0	−67·4	−52·1
3·4	−10·1	− 9·0	9·0	−72·1	−55·7
3·6	−16·5	−15·1	9·5	−74·0	−57·6
4·0	−27·4	−23·2			

Effect of Normal and Immune Sera on the Cataphoretic Velocity.

The effect of normal and immune sera of rabbits on the cataphoresis of *Leishmania tropica* suspended in an aqueous solution of $M/200$-phosphate-glycerine buffer (p_H 7·4) was determined. The immune serum was prepared by injecting rabbits repeatedly with increasing doses of *Leishmania tropica*. The immune serum thus obtained gave agglutination up to a dilution of 1 in 8000. The results are recorded in Table III. It will be noticed that the immune serum diminishes the cataphoretic velocity and hence the electrokinetic potential to a much greater extent than the normal serum. At a dilution of one in two thousand, the normal serum affects the

cataphoretic velocity very slightly, changing it from 59·9 to 58·8 cm. only; while the immune serum decreases it to a marked extent from 59·9 to 46·2 cm.

TABLE III.

Cataphoretic velocity in the absence of any serum = 59·9 cm.

Serum dilution.	Normal serum. (*V*).	Immune serum (*V*).	Serum dilution.	Normal serum (*V*).	Immune serum. (*V*).
1/100	34·7	26·3	1/1200	51·2	38·5
1/200	37·5	28·6	1/1600	55·7	43·1
1/400	43·1	31·6	1/2000	58·8	46·2
1/800	48·5	35·0			

Effect of Hydrogen Ion Concentration on the Cataphoretic Velocity of Sensitised Leishmania Tropica.

The effect of varying the concentration of hydrogen ions on the cataphoretic velocity of *Leishmania tropica* treated with 1/1000 immune serum was also determined and the results are recorded in Table IV. Citrate-phosphate buffers were used in this set of experiments. It will be noticed that in the neighbourhood of p_H 5·3, the cataphoretic velocity of the sensitised micro-organisms is zero. Above p_H 5·3, they are negatively charged and below p_H 5·3 they are positively charged. This shows that the sensitised protozoal bodies have got a protein coating on the surface and the iso-electric point of this adsorbed antibody protein is in the neighbourhood of p_H 5·3.

TABLE IV.

p_H.	*V*.	p_H.	*V*.
8·0	−43·5	5·0	+18·5
7·0	−37·1	4·6	+23·5
6·0	−25·0	4·0	+28·7
5·6	−14·6	3·0	+34·2

We offer our grateful thanks to Dr. J. C. Ray, Director, Indian Institute for Medical Research for kindly supplying us with the *Leishmania tropica* suspensions.

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EFFECT OF HYPERTHYROIDISM ON THE METABOLISM OF VITAMIN C.

By S. N. Ray.

The amount of vitamin C required by guinea-pigs for complete protection is greater in the case of animals fed on desiccated thyroid. In rats, thyroid feeding has no effect on the concentration of ascorbic acid in liver or suprarenals but the concentration of the vitamin in kidney is greatly diminished. This diminution is roughly proportional to the amount of thyroid ingested by the animals.

In recent years, a number of papers have been published trying to establish a definite relationship between vitamin C requirement and the metabolic activity. Thus Harrison (*Biochem. J.*, 1933, **27**, 1501) and Euler and Klussmann (*Z. physiol. Chem.*, 1933, **219**, 215) showed that tissue slices from scorbutic animals have a low oxygen uptake and Soderstrom and Tornblom (*Skand. Arch. Physiol.*, 1933, **66**, 67) have shown that the oxygen uptake of animals is lowered in scurvy. Ecklen and Kooy (*Acta Brev. Neerland Physiol.*, 1934, **3**, 169) showed that when rats were fatigued by long exercise on a treadmill, the ascorbic acid in the suprarenal and in the liver fell to a low level. Abbasy, Harris, Ray and Marrack (*Lancet*, 1935, 1399) also found that the vitamin C output in the urine was much diminished during fever. These results, which indicate a proportionality between vitamin C requirements and metabolic rate, are supported by the fact that the vitamin is usually found in places where active growth is taking place.

This work was started in order to collect more information on this question. The object was essentially to establish a more quantitative relationship between vitamin C intake and the metabolism of the body taken as a whole. The results obtained fully confirmed the theory that the vitamin C requirement is proportional to the metabolic rate. Other interesting results were also obtained and the experimental findings are detailed below.

After various trials the administration of desiccated thyroid to the experimental animals was found to be the most suitable means of increasing the metabolic rate. The other recognised method, *i.e.*, forced exercise was not found suitable. Guinea-pig weighing from 250 to 300 g. were kept on a basal diet of oats, bran, dried egg-yolk and salt-mixture. To provide sufficient vitamins A and D, two drops of cod-liver oil were given to each animal per day. The animals were divided into several groups. Some of the groups were given ascorbic acid in addition to their basal diet, while others were given the basal diet supplemented with desiccated

thyroid and ascorbic acid. One group served as the negative control and was given the basal diet alone. After fourteen days all the animals were killed and cross-sections of the roots of the incisors were prepared by the usual method. The degree of protection as measured on the Key and Elphick's scale (*Biochem J.*, 1931, **25**, 888) gave directly the antiscorbutic activities of the supplements in each group. The results are given in Table I.

TABLE I.

Supplement.	Degree of protection. (complete protection = 4)
100 Mg. desiccated thyroid + 1 mg. ascorbic acid	2, 0, 0, 1 (aver. 0·75)
100 Mg desiccated thyroid + 2 mg. ascorbic acid	1, 2·5, 2·5 (aver. 2)
1 Mg. ascorbic acid only	1, 2, 2 (aver. 1·6)
2 Mg. ascorbic acid only	3, 3, 4, 4 (aver. 3·5)
Basal diet alone	0, 0·5 (aver. 0·25)

These results demonstrate that the amount of vitamin C usually necessary for protecting the animal from scorbutic symptoms becomes insufficient for the proper maintenance of animals whose metabolic rate has been increased through the administration of thyroid.

It was decided also to examine the effect of the thyroid administration to rats. These animals are known to live for indefinite periods on a strictly vitamin C-free diet and the assumption is made that these animals can synthesise their own vitamin C. As has already been mentioned, Ecklen and Kooy (*loc. cit.*) had found that forced exercise in rats reduced the ascorbic acid content of their livers and suprarenals. It was naturally thought that perhaps the same result would be obtained with thyroid feeding. But surprisingly enough it was noticed that in rats to which moderately large doses of desiccated thyroid were administered, the vitamin C content of the livers and suprarenals did not show much variation from similar tissues of rats kept on a scorbutic diet. One marked discrepency was, however, seen in the ascorbic acid content of kidney, the concentration of the acid being much less in the kidneys of the thyroid-fed animals. On repeating the experiment, the same result was obtained and, moreover, it was observed that the diminution in the ascorbic acid content of the kidneys was roughly proportional to the amount of desiccated thyroid fed. The results are given in Table II.

EXPERIMENTAL.

In order to prevent the ingestion of any exogenuous vitamin C, the experimental animals were kept on a strictly scorbutic diet. Guinea-pigs

on such a diet usually survived for three to four weeks and then died of acute scurvy. Mature albino rats were used all through the experiment Weighed amounts of desiccated thyroid were given to the animals every morning. In order to ensure that the whole amount was eaten by the rats, the food pots were removed from the cages during the night, so that the animals used to be very hungry in the morning. The doses used were invariably lapped up clean by the animals. The food pots containing the basal diet were then returned to the cages. After five days all the animals were killed, their tissues removed and extracted with 20% trichloroacetic acid and the ascorbic acid in the extract was determined by titration against a standard solution of 2:6-dichlorphenol-indophenol (Birch, Harris and Ray, *Biochem. J.*, 1933, **27**, 590).

TABLE II.

Supplement.	Amount of ascorbic acid in mg./g. of wet tissue.		
	Liver.	Suprarenals.	Kidney.
Basal diet + 100 mg. desiccated thyroid	0·24	3·8	0·12
Basal diet + 250 mg. desiccated thyroid	0·22	3·2	0·09
Basal diet + 500 mg. desiccated thyroid	0·23	3·4	0·07
Basal diet alone	0·25	3·5	0·23

DISCUSSION.

This diminution of the ascorbic acid content of the kidneys due to thyroid feeding is very interesting. A similar diminution in the concentration of the vitamin in the cerebospinal fluid due to hyperthyroidism had been recently noted by Plant and Bulow (*Klin. Wochschr.*, 1934, **13**, 1744; 1935, **14**, 276, 1318; *Z. Ges. Neurol. Psychiatr.*, 1935, **152**, 84, 1324). On the basis of the theory that the vitamin C requirement of an animal is proportional to its metabolic activities, the ascorbic acid content of all the tissues should have been expected to diminish in case of increased metabolism. But the preferential using up of the vitamin in the kidneys alone makes the question more complicated. One possible explanation of this phenomenon seems to be that thyroid feeding stimulates the processes of oxidation in the kidney cells, whereas such processes in liver or suprarenals are not disturbed. The peculiar fixed limit of the effect on normal oxidation—40% under thyroid control—suggests also control of specific reactions rather than an uncontrolled catalysis.

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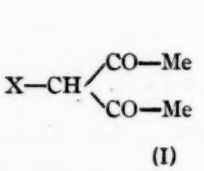
QUINOLINE DERIVATIVES. PART V.

By Tejendra Nath Ghosh.

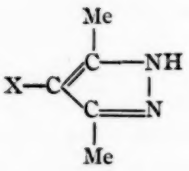
The synthesis of triazolylquinoline is described.

1:5-Diphenyl-3-hydroxy-1:2:4-triazole has been found to have antipyretic activity (Pietra, *Arch. Int. pharmacodynamie*, 1929, **36**, 236), although it is rather toxic. It, therefore, seemed of some interest to synthesise a 1:2:4-triozolylquinoline derivative, which is expected to possess antipyretic activity.

Diacetylthioacetocarbamic acid readily reacts with hydrazine hydrate or phenylhydrazine to yield a 1:2:4-triazole derivative of the type (I) (*cf.* Ghosh, *J. Indian Chem. Soc.*, 1936, **13**, 88). When allowed to react further with hydrazine hydrate, the compound (I) furnishes the triazolyl pyrazole derivative (II). The anilide (III), obtained by condensing (I) with aniline, furnishes the triazoiylquinoline derivative (IV), when treated with strong sulphuric acid at 110°.



(I)



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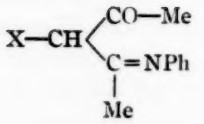
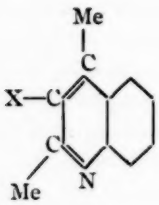
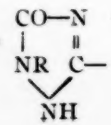


(III)



(IV)

where X= 

EXPERIMENTAL.

2-Phenyl-3-keto-5-diacetylmethyl-dihydro-1:2:4-triazole (I, R=Ph).—Phenylhydrazine (5·4 g.) was added to an alcoholic solution of diacetylthioacetocarbamic acid (10·2 g.), when the reaction immediately commenced with evolution of sulphuretted hydrogen and rise in temperature. For completion of the reaction, the reaction mixture was heated under reflux for 1½ hours. When cooled, the solution was diluted with water, when a semi-solid mass was obtained. It was triturated with cold dilute hydrochloric acid and filtered. The solid crystallised from dilute alcohol in colourless rectangular plates, m.p. 105-106°, yield 7 g. (Found: C, 60·64; H, 5·48; N, 16·38. $C_{13}H_{13}O_3N_3$ requires C, 60·23; H, 5·01; N, 16·21 per cent). It is insoluble in cold dilute alkali.

The acid solution was treated with excess of sodium bicarbonate, when a pasty mass was obtained, which did not solidify even when kept in a vacuum desiccator over concentrated sulphuric acid for a long time and could not be crystallised. It is probably the condensation product of (I, R=Ph) with another molecule of phenylhydrazine.

δ-Phenylimido-γ-(2-phenyl-3-ketodihydro-1:2:4)-triazolyl-β-ketopentane (III, R=Ph).—A mixture of aniline (3 g.) and the above compound (I, R=Ph; 8·6 g.) was heated on an oil-bath at 160-70° for 2 hours, when there was elimination of water vapour. The cold molten mass was triturated with alcohol, when a solid was obtained which was washed with ether and crystallised from alcohol in colourless rectangular plates, m.p. 205-207°, yield 5 g. (Found: C, 68·43; H, 5·77; N, 16·52. $C_{19}H_{18}O_2N_4$ requires C, 68·26; H, 5·38; N, 16·76 per cent).

2:4-Dimethyl-3-(2′-phenyl-3′-ketodihydro-1′:2′:4′)-triazolylquinoline (IV, R=Ph).—A solution of the above compound (III, R=Ph; 3 g.) in concentrated sulphuric acid (15 c.c.) was heated on an oil-bath at 110° for one hour, cooled and poured into ice-cold water. The precipitated solid was filtered and after crystallisation from alcohol was found to be the original compound (III, R=Ph). The clear acid solution was treated with excess of sodium bicarbonate, when a solid was obtained, which was purified by dissolving in dilute hydrochloric acid and precipitating with excess of sodium bicarbonate. It crystallised from alcohol in colourless needles, melting completely at 295° to a thick reddish brown syrup, yield 1·5 g. (Found: C, 71·79; H, 5·47; N, 17·44. $C_{19}H_{16}ON_4$ requires C, 72·15; H, 5·06; N, 17·72 per cent). It is insoluble in dilute alkali but readily soluble in cold acid.

2:4-Dimethyl-3-(3′-ketodihydro-1′:2′:4′)-triazolylpyrazole (II, R=H).—An alcoholic solution of equimolecular proportions of hydrazine hydrate and

the compound (I, R=H; prepared according to the method of Ghosh, *loc. cit.*), was heated under reflux for about 3 hours, and then diluted with water until the solution was slightly turbid and allowed to cool. The crystalline solid, thus obtained, was further crystallised from dilute alcohol in colourless rectangular plates, m.p. 160°. (Found: N, 38·78. $C_7H_9ON_5$ requires N, 39·10 per cent). It is readily soluble in cold dilute alkali and also in cold dilute hydrochloric acid. It does not condense with phenyl *iso*thiocyanate, indicating the absence of any hydrazino group.

δ-Phenylimide-γ-(3-ketodihydro-1:2:4)-triazolyl-β-ketopentane (III, R =H).—A mixture of aniline (3 g.) and the compound (I. R=H; 6 g.) was heated on an oil-bath at 150-55° for about 2 hours, when there was elimination of water vapour. The temperature was raised to 180° for about 15 minutes and the molten mass allowed to cool. It was triturated with cold alcohol, when a solid was obtained which crystallised from alcohol in colourless needles, m.p 226-27°, yield 3·5 g. (Found: N, 21·39. $C_{13}H_{14}O_2N_4$ requires N. 21·70 per cent). It is insoluble in hot concentrated hydrochloric acid and gives a brownish red colouration with ferric chloride.

The above compound was heated with concentrated sulphuric acid at 100° for 30 minutes. The solution was cooled and poured into ice-cold water, when no precipitate was obtained even on long standing. When treated with excess of sodium bicarbonate, the solution remained clear and did not deposit any precipitate. Nothing could be obtained by treating the clear solutions with ether.

My thanks are due te Professor P. C. Guha for his kind interest in this investigation. My thanks are also due to the Lady Tata Memorial Trust for the award of a scholarship.

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STUDIES IN INDIAN BAUXITE. PART I. CHROMIUM AND VANADIUM.

By S. C. Ganguli and J. Das-Gupta.

Cr_2O_3 and V_2O_5 contents of fifteen samples of Indian bauxite have been described in the following paper.

Owing to the importance both from academic and from industrial points of view, bauxite has been the subject of much study. Extensive information on the subject and a very good bibliography will be found in the monograph by Ceril Fox ("Bauxite and Aluminous Laterite", Crossby, Lockwood and Sons, London, 1932).

From time to time, many minor constituents of bauxite from various countries have been reported—Ni, V. Cr etc. in Hungarian bauxite (Gedeon, *Magyar. Chem. Foloy*, 1932, **38**, 134); chromium and vanadium in Australian bauxite (Fox, *loc. cit.* p. 143), and so on. So far as the present authors are aware, no investigation on the minor constituents of Indian bauxites have been carried out except by Dr. Bruhl in his analysis of Kalahandi bauxite. The present investigation was undertaken to study the minor constituents of the Indian bauxites and their chromium and vanadium contents are reported in this paper.

Experimental.

In general the method followed for estimation was similar to the one described by Sandell (*Ind. Eng. Chem. Anal. Ed.*, 1936, **8**, 336). But two distinct methods were used to obtain *the sum* of the total quantities of oxides.

Method I.—Bauxite (1·5—2 g.) was fused with Na_2CO_3 and KNO_3 and the melt was extracted with water and filtered. The p_H value of the filtrate was adjusted to 7·0 with the requisite quantity of H_2SO_4 when all the aluminium was precipitated as hydroxide. After the separation of aluminium, chromium and vanadium were precipitated as mercurous salts, and the precipitate was ignited. The weight of the ignited precipitate gave the value of $Cr_2O_3 + V_2O_5$. The mixed oxides were then fused with Na_2CO_3, dissolved in water, and chromium and vanadium were estimated in the solution by the colorimetric method (Sandell, *loc. cit.*).

Method II.—The sodium carbonate extract was just neutralised with 4*N*-sulphuric acid and chromium and vanadium were estimated by the colorimetric method of Sandell without the previous separation of alumina.

The results obtained from both the methods agreed within experimental error. Before powdering a sample of bauxite, all extraneous matter which were considered not to be bauxite or highly contaminated bauxite, were excluded by hand picking.

Bauxite No.	Locality.	Method I.		Method II.	
		V_2O_5.	Cr_2O_3.	V_2O_5..	Cr_2O_3.
134	Netarlvat, Palamau, Bihar	0·074%	0·064%	0 078%	0·060%
737	N. E. of Amarkantak. S. Rewah	0·080	0·14	0·0865	0·160
797	Yelurgarh fort, Bombay	0·092	0·125	0·086	0·125
291	Salgipat, N. Lohardaga, Bihar	0·062	0·030	0·067	0·025
790	Radhanagri, Kolhapur, Bombay	0·760	0·062	0·0766	0·060
788A	N. W. of Radhanagri	0·125	0·100	0·119	0·107
978	West of Pipagarh, Balaghat Dist C.P.	0·142	0·055	0·130	0·056
1128	Surganj State	0·058	0·060	0·064	0·056
1142	,,	0·115	0·051	0·103	0·051
1559	Yeruli Pleateau, Bombay	0·089	0·108	0·914	0·102
4696	Katni, Jubbulpore, C.P.	0·067	0·048	0·059	0.054
5038	1 Mile N. W. of Dhagarvadi, Kolhapur, Bombay	0·043	0·056	0·049	0·058
5097	Kapadvaraj. Khaira Dist., Bombay	0·028	0·109	—·—	——
5098	Jubbulpore, C.P.	0·098	0·088	0·108	0·884
5100	Salal, Riasi Tehsil, Kashmir	0·0025	0·013	——	——

From the above data it will be seen that though many samples of bauxite from peninsular India contain appreciable amounts of chromium and vanadium, the sample from Kashmir (No. 5100) contains only a small quantity in comparison with them. No conclusion on the matter is possible without further and more representative examination.

The authors desire to thank the Geological Survey of India for kindly supplying the samples of bauxite and Dr. P. B. Sarkar for suggesting the subject and guidance. Their best thanks are also due to Prof. N. N. Sen for affording all possible laboratory facilities and for his kind interest.

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CATALYTIC FORMATION OF METHANE FROM CARBON MONOXIDE AND HYDROGEN. PART V. A STUDY OF THE PROMOTER EFFECT UPON NICKEL CATALYST.

By K. M. Chakravarty.

The reduction of nickel catalyst at a temperature of 300° is incomplete when prepared from a very pure sample of nickel acetate. Addition of a small quantity of a mixture of cerium nitrate and ammonium vanadate or of cerium nitrate and chromium acetate to the nickel acetate helps in bringing about a more thorough reduction Thus catalysis of the reaction $2CO+2H_2=CO_2+CH_4$ is directly accelerated by them. Whether there is any direct beneficial effect of the promoters cannot be said at this stage.

Very often small quantities of promoters are added to increase the activity of a catalyst. It is believed that the joint effect of the catalyst material and the promoters somehow accelerates a reaction to a greater extent than if the catalyst were used alone. But up till now very little work has been done to show that these added promoters influence the actual physical and chemical processes through which the catalyst passes in course of its preparation, apart from their direct effect if any upon the reaction which the catalyst is to accelerate. It may be, that in certain cases, they influence both the condition of preparation of the catalyst as well as its direct action. But there may also be cases where only one of these factors is operative. Again a substance, which favourably affects the one, may have an adverse influence upon the other. Ingredients which adversely affect the former will yield, however, a catalyst of low activity or of none. In this case, the poisoning effect on the catalytic reaction is only indirect. In the same way the promoter effect may be only an indirect one. It, therefore, follows that a theory of promoter action and of poisoning cannot be correctly developed unless one knows which of the above factors is actually affected by the promoter or poison, as the case may be.

Armstrong and Hilditch (*Proc. Roy. Soc.*, 1923, **A103**, 586) have shown that the nickel catalyst containing more than 5% of alumina did not catalyse the hydrogenation of oil. It was shown that this inactivity was due to imperfect reduction of the catalyst. Evidently here alumina directly poisoned the reduction of nickel oxide and hence indirectly the hydrogenation. It also follows from the above cases that when a metallic surface is the catalyst, a partially reduced surface will be inactive or will possess

only a lower activity. Similarly where a lower oxide is the catalyst, a higher oxide will not be of much use and so on.

Fischer and his co-workers (*Brennstoff. Chem.*, 1933, **14**, 64) got good nickel catalyst by carrying out reduction with a mixture of nitrogen and hydrogen in one case and ammonia and hydrogen in the other. Rogers (*J. Amer. Chem. Soc.*, 1933, **49**, 1432) showed that in presence of copper, zinc oxide was reduced to the metal at a temperature where it was not attacked when alone. Reduction of zinc oxide to zinc in presence of copper was also found by Frollich and his co-workers (*Ind. Eng. Chem.*, 1929, **21**, 109).

A nickel catalyst is generally known to catalyse reaction

$$2CO + 2H_2 \rightleftharpoons CH_4 + CO_2.$$

We have also seen the inhibiting action of 5% alumina on the reduction of nickel catalyst in Armstrong's investigations cited before. The following investigation will show how far the promoters accelerate the chemical reactions that occur during the preparation of nickel catalyst and thereby indirectly its activity towards the above reaction.

EXPERIMENTAL.

Nickel acetate was very carefully prepared so as to avoid, as far as practicable, the presence of any poison or promoter. A known quantity of the acetate with or without a promotor was dissolved in water and deposited on purified and ignited pumice (10-20 meshes). The dried product was then introduced inside a glass tube and was softly packed between two plugs of asbestos fibre A thermometer was inserted inside the catalyst mass to read the temperature during the experiments. This tube containing the catalyst was then introduced into an electric resistance furnace. The catalyst was reduced in a current of hydrogen and next studied *in situ* with an 1 : 1 (by volume) mixture of carbon monoxide and hydrogen. The experimental details were practically the same as described in Part I of this series of papers (*J. Indian Chem. Soc.*, 1925, **2**, 150).

Catalyst I : Pumice (17 g.), nickel acetate (3·5 g.).

Catalyst II : Pumice (17 g.), nickel acetate (3·5 g.), cerium nitrate (0·06 g.), vanadic acid* (0·04 g.).

Catalyst III : Pumice (17 g.), nickel acetate (3·5 g.), cerium nitrate (0·06 g.), chromium acetate (0·25 g.).

* Ammoniacal solution of 0·04 g. of vanadium pentoxide was used.

TABLE I.

Temp. of reduction = *300°.

Catalyst No.	Space velocity.	Vol. comp. of the resultant gas mixture.				Catalyst temp.	Remarks.
		CH_4.	CO_2.	CO.	H_2.		
I	0·44	3%	0·25%	50·3%	46·2%	†370°	These experiments were repeated three times for confirmation
II	1·4	37 7	28·8	25·5	11·0	300	
III	2·7	44·9	30·7	17·7	7·3	,,	

DISCUSSION.

From the data given in Table I, it is clear that nickel catalyst without any promoter is practically inert so far as the reaction

$$2CO + 2H_2 \rightleftharpoons CH_4 + CO_2$$

is concerned. Additions of promoters have increased the activity considerably. The point arises as to whether the promoters have got any direct beneficial effects on the above reaction.

Although all the catalysts were reduced at the same temperature, the colour of the pure nickel catalyst after reduction was grey-black with a greenish ting. The colour of the promoted catatysts after reduction was black. This clearly indicates that the extent of reduction in case of pure nickel catalyst was less in comparison with those containing the promoters. Armstrong and Hilditch (*loc. cit.*) have shown that colour is black when the catalyst contains 80 7—44% of metallic nickel. It is grey-black when nickel present is 4·5%.

In the present investigation, the catalysts were not actually analysed to estimate the extent of reduction. For it was felt that such data, though valuable to get an idea regarding the average composition of the catalyst mass, do not indicate the correct composition of the surface. For it is not possible to analyse a surface. To get an idea regarding the nature of the surface, the catalyst material after its activity was studied with carbon monoxide and hydrogen mixture was allowed to cool in an atmosphere of this gas mixture. Nickel deposition was seen to occur only in case of the active catalysts. With a pure nickel preparation no such deposition occurred. Certainly sufficient metallic nickel particles were present on the catalyst surface containing promoters The colour of the pure nickel

* No reaction occurred at 300°.

† The excesss of hydrogen is due to that already present in the reaction tube.

catalyst combined with the analytical data of Armstrong and Hilditch and the fact that it did not form nickel carbonyl, clearly indicates that the surface was only partially reduced.

Schmidt (*Chem. Rev.*, 1933, **12**, 371) has shown particularly in case of finely divided nickel (*Z. physikal. Chem.*, 1925, **118**, 216) that the progress of reduction is considerably hindered by sintering. Thereby inertness is produced and a part of the oxide remains unreacted upon. It is possible that the pure nickel catalyst was sintered in the intermediate stage and the promoters pushed the reduction to completion probably by stopping this sintering.

From the experimental data and what has been said above it cannot be said definitely whether the promoters had any direct accelerating influence upon the reaction

$$2CO + 2H_2 \rightleftharpoons CH_4 + CO_2$$

but it is clear that they helped a good deal, the process of reduction of the catalyst, thereby increasing the number of nickel particles on its surface.

I take this opportunity of expressing my thankfulness to Prof. J. C. Ghosh for kindly offering me all facilities for work and for the encouragement which I received from him constantly. I thank Prof. S. N. Bose for the interest which he so kindly took in this investigation.

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FURTHER STUDIES ON THE HYDROLYSIS OF STARCH BY SWEET POTATO AMYLASE.

By K. V. Giri.

The nature of the hydrolysis of amyloamylose and soluble starch by sweet potato amylase and β-amylase of barley is investigated. The sweet potato amylase behaves like a pure β-amylase. The amylase selectively hydrolyses a portion of the starch substance, leaving a residual material which resembles erythrogranulose. Amyloamylose is hydrolysed completely by both the enzymes, without noteworthy inhibition, and the colour with iodine remains unchanged until almost complete saccharification takes place. During the saccharification of amyloamylose by sweet potato amylase the intensity of the blue colour with iodine diminishes. The residual material is hydrolysed by α-amylase from barley, but it remains unaffected by sweet potato amylase.

It would appear from the study of starch chemistry that the only line of enquiry which promises to be of immense value in the elucidation of the structure of starch is the study of its hydrolysis by the enzyme diastase. In fact the greater part of the knowledge we possess of starch has been derived from a study of its hydrolytic products under the influence of enzymes. The importance of such studies lies not only in the elucidation of the structure of starch but also in the solution of the problem of enzymes and their reactions in general.

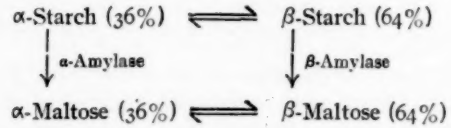
In more recent times fresh light has been thrown on the enquiry by a study of the action of different types of amylases on starch. As a result of such studies, investigators have put forward different views on the amylolytic decomposition of starch.

The Theory of Specificity.

Kuhn (*Annalen*, 1925, **443**, 1) has made the discovery that there are two different types of amylases which are separable under certain conditions. One of these amylases converts starch into α-maltose, the other produces only β-maltose under similar conditions. These observations indicate two possibilities—either (*i*) there are two distinct starches made up of α- and β-maltose units respectively or (*ii*) both the α- and β- forms of glucosidic linkage exist in the starch molecule, either alternately or according to some regular plan so that the starch molecule, when hydrolysed, breaks down at the one or the other centre. It is, however, considered that the two forms of maltose arise from the breakdown of a single substrate through alternative types of hydrolysis.

α- and β-Starches.

The existence of two different specific amylases is a strong point in favour of the existence of corrrespondingly different components in starch molecule. More recently Van Klinkenberg (*Proc. Acad. Sci. Amsterdam*, 1931, **34**, 893 ; *Z. physiol. Chem.*, 1932, **209**, 253 ; *ibid.*, 1932, **212**, 173 ; *Ergebnisse Enzymfors.*, 1934, **3**, 73) has put forward the view that the liberation of α- and β-maltose by α- and β-malt amylase is due, not to alternative types of hydrolysis of a single substrate, but to the selective hydrolysis of two components of starch, which he designated as α- and β-starch. The following scheme represents the view of Van Klinkenberg on the relations between the two starches and the two amylases.



The hypothesis developed by Van Klinkenberg is based on the following points :

(1) When soluble starch is hydrolysed to completion by β-amylase, the reducing power of the products corresponds to 64% of the theoretical maltose. The iodine colour remains blue or blue-violet owing to the presence of non-reducing residual material. This residual fraction resembles Wijsman's " erythrogranulose " or Baker's " α-amylodextrin" (*J. Chem. Soc.*, 1902, **81**, 1177) This fraction is designated as α-starch and constitutes about 36% of the starch.

(2) When soluble starch is hydrolysed by α-amylase the reducing power rises rapidly to a value that corresponds to 36% of the theoretical maltose, after which it continues to rise slowly. The initial rapid rise is interpreted as being due to the selective hydrolysis of α-starch (36% of the total substrate) to maltose. The slow hydrolysis beyond 36% in presence of α-amylase was explained by the supposed slow transformation of β- into α-starch. The converse transformation (α-starch into β-starch) does not occur because a sharp " saccharification limit is always observed in the hydrolysis of starch by β-amylase.

Hanes (*Canadian J. Res.*, 1935, **13B**, 185) by carefully planned experiments has confirmed in essential respects the observation of Van Klinkenberg concerning the action of α- and β-malt amylases. But he has considerably modified the view of Van Klinkenberg by studying the reducing products with the help of yeast for their fractionation. The experiments of Hanes have shown that α-amylase does not selectively hydrolyse the erythrogranulose

fraction of starch, but that from the beginning of the reaction the greater part of reducing material arises from the β-starch fraction. The recent findings of Freeman and Hopkins (*Biochem. J.*, 1936, **30**, 446) also do not support the view of Van Klinkenberg of selective hydrolysis of different components of the starch by the respective amylases. The conclusions drawn from their experiments are as follows.

(1) "Both α- and β-amylases readily attack and hydrolyse completely the amyloamylose component (about 20%) of starch, and a portion of the erythroamylose component. (2) Both enzymes hydrolyse much the same fraction, 60% of the starch; but whereas the remainder (α-amylodextrin) is almost, if not completely, resistant to the β-amylase, it can be with difficulty hydrolysed by the α-enzyme. (3) α-Amylodextrin, the hydrolysis of which has been commenced by the α-amylase, can be further degraded to a certain extent by the β-amylase. The α-enzyme holds the key to further hydrolysis. (4) In degrading starch to the 50% stage, the α-enzyme does so mainly at the expense of the portion which is other than amylodextrin, since action on the residue by β-amylase still leaves about 30% of the original starch unattacked."

Amyloamylose and Erythroamyloses.

According to Samec and Mayer (*Koll.-Chem. Beih.*, 1921, **31**, 272) starch in solution contains certain distinct components which may be fractionated by electrodialysis yielding respectively amyloamylose (blue with iodine) and erythroamylose (red with iodine). Samec and Waldschmidt-Leitz (*Z. physiol. Chem.*, 1931, **203**, 16) have further investigated the hydrolysis of these components by different amylases, and have shown that amyloamylose is hydrolysed by pancreatic (α-), barley (β-) and unfractionated malt (mixture α+β) amylases relatively easily and completely, but erythrogranulose only with difficulty. Thus Van Klinkenberg's so-called α- and β-starches are not identical with amylo- and erythro-amyloses, though they might represent fractions of the erythroamylose, which constitutes at least 60% (according to Freeman and Hopkins, *loc. cit.*) to 83% (according to Samec) of the total starch.

The present investigation was undertaken with a view to elucidating certain aspects of the amylolytic decomposition of starch by sweet potato amylase, and with the primary object of obtaining evidence as to the nature of the amylase.

EXPERIMENTAL.

Substrates.—Zulkowsky's soluble starch was used. *Amyloamylose* was prepared by electrodialysis of 2% potato starch, autoclaved at 120° for

½ hour at 220 volt using platinum electrodes and cellophane as the dialysing membrane (Samec and Mayer, *loc. cit.*).

Preparation of Amylases.

Sweet Potato Amylase.—The amylase was prepared from the tuber by the method previously described (Giri, *J. Indian Chem. Soc.*, 1934, **11**, 339) by precipitation from the aqueous extract of the malt with alcohol. Control experiments under the condition of reaction employed in the experiments indicated the absence of any maltase activity from the amylase preparations.

β-Amylase from Barley.—The enzyme was prepared from ungerminated barley by the method described by Hanes (*loc. cit.*). Ungerminated barley was ground to a fine flour. 200 G. of the flour were extracted with 700 c.c. of 50% alcohol for 2 hours with occasional shaking and centrifuged. The residue was again re-extracted with 700 c.c. of 50% alcohol for 2 hours and centrifuged. The supernatant liquors were combined and after filtration the enzyme was precipitated by adding alcohol to a concentration of 80%. The precipitate was removed by centrifuging, washed with alcohol and ether and finally dried in a desiccator.

α-Amylase from Barley-malt.—Barley seeds were soaked in flowing water for 24 hours and allowed to germinate at 25-28° for 3 days. The seedlings were then dried in the sun and ground to a flour. The preparation of α-amylase from the malt thus obtained was exactly similar to that described by Hanes (*loc. cit.*). 250 G. of the flour were extracted once with 700 c.c. of dilute aqueous solution of potassium hydrogen phosphate solution (0·05%) for 2 hours at about 4-5° and again with 500 c.c. of the phosphate solution for about ½ an hour. The extracts were mixed together and centrifuged to remove any suspended material. Alcohol was then added to the combined extract to a final concentration of 60%. The precipitate was dispersed in 350 c.c. of water and heated to 70° for 15 minutes in order to destroy the activity of β-amylase. After cooling it was centrifuged and filtered. The enzyme was again precipitated by adding alcohol to give a concentration of 65%. The precipitate was washed with 90% alcohol and finally with absolute alcohol and dried in *vacuo* over calcium chloride. The yield was about 0·7 g.

The purity of the preparations of α- and β-amylases as prepared by the above methods was tested by the diffusion method described by Giri (*J. Indian Inst. Sci.*, 1934, **17A**, 127). The results showed that the preparations behaved like pure α- and β-amylases.

The maltose was determined by the method of Willstätter and Schüdel (*Ber.*, 1918, **51**, 780).

1 c.c. $N/10\text{-}I_2 = 17{\cdot}15$ mg. of maltose.

The percentage of hydrolysis is expressed as percentage of maltose (105·5%) theoretically equivalent to the complete conversion of the quantity of substrate used.

The "Saccharification limit" for the Hydrolysis of Starch by Sweet Potato Amylase and Barley β-Amylase.

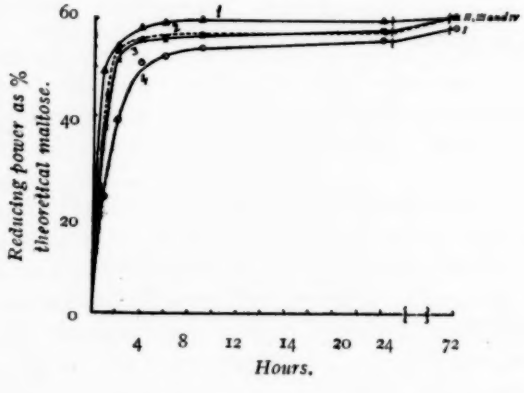
It is well known that when starch is decomposed by amylases inhibition zones and saccharification limits are formed, which are found to vary with different types of amylases, and the results obtained by several workers are found to be of varying magnitude. The following table represents the values given by several workers.

Authors.	Reference.	Inhibition zones.
	α-Amylase.	
Syniewski	... *Annalen*, 1925, **441**, 288.	about 34%
Polak and Tychowski	... *Biochem. Z.*, 1929, **214**, 216.	„ 36%
Van Klinkenberg	... *Z. physiol. Chem.*, 1932, **209**, 253.	„ 36%
Hanes	... *Canadian J. Res.*, 1935, **13B**, 185.	„ 34-38%
Blom, Bak and Braae	... *Z. physiol. Chem.*, 1936, **241**, 273.	„ 33-40%
	β-Amylase.	Saccharification limits.
Baker	... *J. Chem. Soc.*, 1902, **81**, 1177.	60-65%
Ling and Nanji	... *J. Chem. Soc.*, 1923, **123**, 2666; 1925, **127**, 636.	67%
Polak and Tychowski	... *loc. cit.*	66%
Van Klinkenberg	... *loc. cit.*	64%
Hopkins, Cope and Green	... *J. Inst. of Brew.*, 1933, **39**, 487.	64-65%
Hanes	... *loc. cit.*	61%
Blom, Bak and Braae	... *loc. cit.*	53·1 ± 0·5%

The figures in the above table indicate that the hydrolysis limit of the decomposition of starch is reached at 60-67% of hydrolysis of starch. The amylase of resting barley grain is almost pure β-amylase, but a small amount of α-amylase is present in the crude aqueous extracts. In the following experiments the action of β-amylase of barley, prepared according to the method of Hanes on starch was studied and compared with that of the amylase from sweet potato.

All reactions were performed at p_H 4·6 (0·0286*N*-acetate buffer) and p_H 6·0 (0·0286*N*-acetate buffer) for barley and sweet potato amylase activities respectively, the enzyme solutions having been brought to p_H 4·6 and 6·0 by the addition of acetate buffer. The total volume of the reaction mixture amounted to 140 c.c. and the amount of starch present in the reaction mixture was 1750 mg. The progress of hydrolysis was followed by measuring the amount of maltose formed at known intervals of time, controls for the reducing power of the enzyme preparation and of all substrates being deducted. Usually the maltose determinations were carried out on 20 c.c. samples.

FIG. 1.



Curves 1—4 refer respectively to S. P. amylase (digest No. III), barley β-amylase (digest No. IV), S. P. amylase (digest No. II) and S. P. amylase (digest No. I).

All results are expressed as percentage of maltose (105·5%) theoretically equivalent to the complete conversion of the quantity of substrate used. The results are shown in Table I and in Fig. 1.

The figures in Table I indicate that a sharp saccharification limit is reached at 61% conversion. This value appears to be independent of the concentration of the enzyme. Further the data show that there is close agreement between the two figures obtained for the saccharification limits by the β-amylase from barley and sweet potato amylase. Further it is seen from Fig. 1 that the hydrolysis is considerably lowered when about 50% saccharification is reached. This experiment would indicate that the sweet potato amylase is a pure β-amylase.

TABLE I.

Saccharification limits for the hydrolysis of starch by sweet potato amylase and barley β-amylase.

Time.	Hydrolysis as percentage theoretical maltose.			
	S. P. amylase digest No. I =5 c.c. of 0·1%.	S. P. amylase digest No. II =10 c.c. of 1%.	S. P. amylase digest No. III. =20c.c. of 1%.	Barley β-amylase digest No. IV. =10 c.c. of 0·5%.
1 hr.	24·4	38·1	49·0	44·6
2	39·7	51·4	54·2	53·3
4	51·4	55·2	58·0	55·2
6	52·7	55·3	59·2	55·3
9	54·0	56·6	60·0	56·6
24	56·0	58·4	60·4	58·4
48	57·2	60·5	60·6	60·6
72	58·5	60·6	60·6	60·7

Hydrolysis of Amyloamylose by Sweet Potato Amylase and Barley β-Amylase.

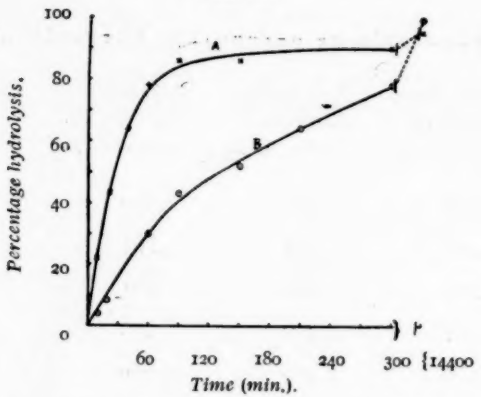
One of the striking characteristic properties of pure β-amylase is that it hydrolyses amyloamylose in such a way that the iodine colour remains blue until about 90% of maltose is formed, then it becomes violet and finally rose (Samec and Waldschmidt-Leitz, *loc. cit.*, Samec, *Z. physiol. Chem.*, 1935, **236**, 103). With a view to finding out whether sweet potato amylase behaves like a pure β-amylase in its action on amyloamylose, the following experiment was conducted.

The hydrolysis of amyloamylose was followed in two digests with sweet potato amylase and barley β-amylase.

Each digest consisted of 250 mg. of amyloamylose, 20 c.c. of $M/5$ acetic acid—acetate buffer (adjusted to p_H 6·0 and p_H 5·2 respectively for sweet potato and barley β-amylase), and 5 c.c. of enzyme extract (0·1% S. P.-amylase and 0·5% barley-β-amylase) in a total volume of 125 c.c. The mixtures were incubated at 35° and the maltose was determined in

10 c.c. samples at frequent intervals. The colouration with iodine was noted by adding 1 c.c. of reaction mixture to 1 c.c. of $N/100$-iodine solution. The results are shown in Table II and Fig. 2.

FIG. 2.



Curves A and B refer respectively to barley-β-amylase and S. P. amylase.

TABLE II.

Saccharification and iodine colouration.

Amyloamylose = 0·25 g. 0·2N-Acetic acid—acetate buffer = 20 c.c.
Total volume = 125 c.c. Temp. = 35°

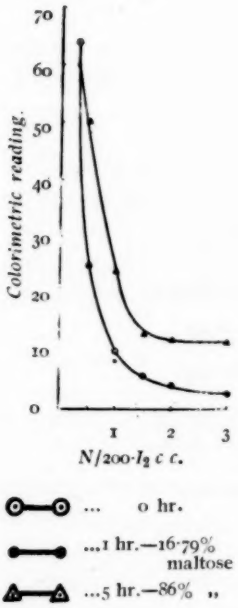
Sweet potato amylase			Barley-β-amylase		
Time	Saccharification in % maltose.	Colour with I_2.	Time.	Saccharification in % maltose.	colour with I_2.
10 min	4·3	Blue	10 min	22	Blue
20	8·5	,,	20	43	,,
60	30	,,	40	64	,,
90	43	,,	60	78	,,
150	52	,,	90	86	,,
210	64	,,	150	87	,,
300	78	,,	300	90	Bluish-green
1440	99·1	Violet	1440	95·2	Violet

It is clear from the results of this experiment that the action of sweet potato amylase on amyloamylose is very similar to that of β-amylase of barley. This experiment in conjunction with the value obtained for the saccharification limit, thus gives ground for the statement that sweet potato amylase is a pure β-amylase. Further the results would indicate that amyloamylose undergoes saccharification to theoretical completion without noteworthy inhibition.

Changes in the Iodine Colouration during the Hydrolysis of Amyloamylose by Sweet Potato Amylase.

In a previous communication (Giri, *Science*, 1935, **81**, 343) it was reported that the colour reactions produced with iodine by different starches in the course of hydrolysis differ markedly with the kind of starch and also with the concentration of iodine used. This may possibly be due to the presence of amylo- and erythroamyloses, the two most important components of the starch, in various starches in different proportions. With a view to throw more light on the behaviour of the starches in the course of hydrolysis towards iodine, the following experiment was carried out with amyloamylose which can be considered as a uniform substrate unlike starch which is a heterogeneous substrate, in the sense that it contains number of components which differ in their behaviour towards iodine. In a recent communication Samec (*loc. cit.*) has also shown that the behaviour of starch substance towards iodine changes during amylolysis in different ways. It changes (1) the colour tone and (2) the colour intensity. He has shown that the hydrolysis of amyloamylose with β-diastase from barley does not bring about any definite change in the iodine colour even when after high saccharification (90% maltose) stage is reached. The blue forming function of starch, however, goes on diminishing in the course of hydrolysis, and for the attainment of maximum colour, smaller quantities of iodine are required, with the increase in saccharification.

FIG. 3.



The following experiment was carried out for the purpose of determining whether sweet potato amylase behaves in a like manner in the hydrolysis of amyloamylose. The change in the colour intensity with iodine in the course of hydrolysis of amyloamylose by sweet potato amylase was determined as outlined below.

The reaction mixture (total volume 180 c.c) consisted of 20 c c. of $M/5$, acetate buffer at p_H 5·5; 150 c c. of substrate containing 495 mg. of amyloamylose, and 10 c.c. of 0·1% sweet potato amylase. Two sets of such digests were prepared. The digests were incubated at 35°. At known intervals of time, 40 c.c. of the reaction mixture were removed. A series of 50 c.c. flasks containing known amounts of $N/200$-I_2 solution and diluted with water was arranged. As soon as the aliquot (40 c.c.) from the reaction mixture was removed, 5 c.c. of the mixture were added into each flask, made up to the mark with water and the colour was compared in a colorimeter against a standard. The readings thus obtained were recorded. Simultaneously the percentage of maltose formed was also determined.

Standard for Colorimetry.—5 C.c. of the original amyloamylose solution (containing 330 mg. in 100 c.c.) were added into a 50 c.c. flask which contained 1 c.c. of $N/200$-I_2, diluted with water, and made up to 50 c c. This was used as standard.

The results are presented in Table IIIA and graphically in Fig. 3, in which the quantity of iodine added is expressed along the abscissa and the thickness of the iodine-starch solution (in the present case the direct colorimetric scale reading) as ordinate. In Table III B are given the percentage of maltose formed in the same reaction mixture at known time intervals.

TABLE IIIA.

Variation in the intensity of the blue colour obtained with iodine during the hydrolysis of amyloamylose by sweet potato amylase.

No.	$N/200$-I_2 added.	Colorimetric reading against the standard after hydrolysis.			
		0 hour.	1 hour.	3½ hours.	5 hours.
1	0·3 c.c.	64	60	70	Colourless
2	0·5	25	25	27	40
3	1·0	10	9·8	10·3	24
4	1·5	5·3	5·9	6·0	13
5	2·0	4·2	4·2	4·3	12·3
6	3·0	2·6	2·8	3·9	11·8

TABLE IIIB.

Percentage of theoretical maltose (105·5 %) formed in the hydrolysis of amyloamylose by sweet potato amylase.

Time.	Maltose formed in the total vol. of reaction mixture.	Colour with 1 c.c. of N/200-I_2.
1 hr.	16·7%	Blue
3½	60	Blue
5	86	Blue
20	88	Blue
44	96	Violet

It will be seen that the property of the starch responsible for the formation of blue colour with iodine diminishes with the increase in saccharification. Further the concentration of iodine required to attain the maximum colour also diminishes with increase in saccharification. The results obtained with sweet potato amylase are thus in agreement with those of Samec in regard to the general behaviour of β-amylase on amyloamylose.

On the Residual Material Resulting from the Hydrolysis of Starch by Sweet Potato Amylase.

Previous workers (Van Klinkenberg, Hanes and others) have shown that when β-amylase acts on starch, the hydrolysis does not go to completion. A fraction resembling Wijsman's erythrogranulose, or Baker's α-amylodextrin, which is designated as α-starch by Van Klinkenberg and which constitutes about 36 % of the starch, is left behind unattacked. According to Van Klinkenberg this residual material does not possess any reducing action and it gives a purple or blue colour with iodine. β-Amylases are considered to be specific for β-starch which constitutes about 64 % of the starch. Erythrogranulose is not attacked by β-amylase. But on prolonged treatment with large amounts of β-amylase, traces of reducing material are formed, and the iodine colour remains unchanged. It is readily hydrolysed by α-amylase with the production of reducing material, and the iodine colouration undergoes a sequence of changes from blue-violet through rose and brown and leading finally to complete absence of colouration.

In the following experiment the preparation and properties of the residual material left after the hydrolysis of starch by sweet potato amylase is reported.

The Preparation of the Residual Material from Soluble Starch by Sweet Potato Amylase Hydrolysis.

The digestion mixture contained 500 c.c. of 9% starch, 50 c.c. of acetate buffer ($N/5$) of p_H 5.5 and 50 c.c. of 0·1% sweet potato amylase solution. After 48 hours' digestion at 37° the mixture gave violet colour with iodine. On boiling the digestion mixture, the slight turbidity disappeared and it became transparent. After cooling the mixture gave blue colour with iodine, thereby indicating that the groups responsible for the production of blue colour with iodine are liberated on boiling. 20 C.c. more of the enzyme solution were again added to the digest and incubated at 37° for 24 hours. The reaction with iodine was again tested and it was found that it gave a violet colour with iodine. Once again 20 c.c. more of the enzyme were added and digested for 48 hours, the total period of digestion amounting to 120 hours. At the end of this period the digestion mixture was boiled to destroy the enzyme and after cooling the colour with iodine was tested and found to give violet-red colour. The reducing power determined on 1 c.c. samples was about 60% of the theoretical maltose.

The residual material from the digestion mixture was precipitated by the addition of 1500 c.c. of 95 % alcohol to 550 c.c. of the digestion mixture. The precipitate was allowed to settle by keeping it at laboratory temperature for about 4 hours. The top layer of liquid was decanted. The residual mass formed at the bottom of the beaker was washed with 70 % alcohol and then with absolute alcohol and filtered through a Buchner funnel. It was then washed with ether and kept for drying in a desiccator. The residual material thus obtained was slightly gummy and transparent in appearance. For further examination the solution of the residual material in water was used.

TABLE IV.

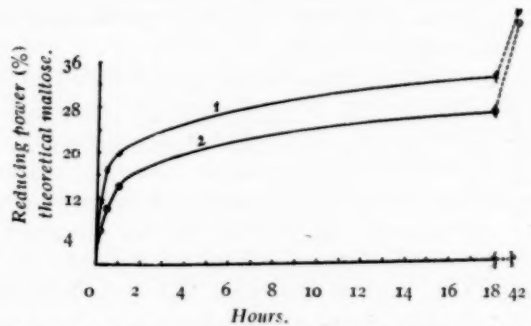
Hydrolysis of the residual material by sweet potato amylase and α-amylase of barley malt.

Digest No. I	25 C.c. of the residual material containing 325 mg. of the substance. 20 C.c. of acetate buffer of p_H 5·5. 10 C.c. of 0·1 % sweet potato amylase. 25 C.c. of water.
Digest No. II	25 C.c. of the residual material. 20 C.c of acetate buffer $N/5$ of p_H 5·2. 1 C.c. of 0·5 % α-amylase from barley malt. 24 C.c. of water.
Digest No. III	Same as digest No. II except that 2 c.c. of the α-amylase were added.

The total volume of all the digests was adjusted to 80 c.c. and digested at 35°. 10 C.c. of the digests were removed at stated intervals and the reducing power was determined. Simultaneously the colouration with iodine was also determined. The results are presented in Table V and Fig. 4.

It will be seen that α-amylase does not completely hydrolyse the residual fraction. The results show that it is hydrolysed to less than 50% of the theoretical amount of maltose. On the other hand the residual material is hydrolysed with great difficulty by sweet potato amylase.

FIG. 4.



Curves 1 and 2 refer respectively to 2 c.c. and 1 c.c. of α-amylase.

TABLE V.

Hydrolysis of the residual material by sweet potato amylase and α-amylase of barley malt.

	Sweet potato amylase.		α-Amylase from barley malt.			
	Digest No. I.		Digest No. II.		Digest No. III.	
Time.	Reducing power as theoretical maltose.	Colour with I_2.	Reducing power as theoretical maltose.	Colour with I_2.	Reducing power as theoretical maltose.	Colour with I_2.
15 min.	0	Violet-red	6·1	Red	11	Colourless.
30	0	,,	10·2	Colourless	17·1	,,
60	0	,,	14·0	,,	20·2	,,
1080	7·2	,,	28·0	,,	34·1	,,
2520	10·2	Red	44·0	,,	46·6	,,

In the case of α-amylase the iodine colouration disappears when less than 10% of maltose are formed in the digest.

DISCUSSION.

The present study has thrown light on the nature of sweet potato amylase and the constitution of starch. In previous communications, it was shown that the enzyme behaves like pure β-amylase. The present investigation has further strengthened this view. Thus the saccharification limit for the hydrolysis of starch by sweet potato amylase corresponds to the value obtained with β-amylase of barley. Further the nature of its hydrolysis of amyloamylose is characteristic of pure β-amylase.

The residual material obtained after the hydrolysis of starch by sweet potato amylase appears to resemble the erythrogranulose fraction of starch in its hydrolysis by α-and β-amylases.

According to Van Klinkenberg the starch is composed of two components, the one (α-starch = 36% of total quantity) producing colour with iodine, and the other (β-starch = 64%) which does not give any colour with iodine. By the hydrolysis with α-amylase, the α-starch is hydrolysed, and the β-starch is transformed into α-form, which is further hydrolysed by the enzyme. In this manner the iodine reaction disappears before the saccharification is complete. The conversion into β-starch is, however, very difficult, and hence the limit of β-amylase hydrolysis has been found to occur when 64% of saccharification has taken place. Thus according to Van Klinkenberg the iodine colouring atomic groups are present in α-starch only and the iodine reaction, therefore, only disappears by the action of α-amylase. The experiments described in the present paper appear to render this view untenable. It was observed that the blue colour obtained with iodine by amyloamylose diminishes during hydrolysis by β-amylase from sweet potato. Therefore β-starch also should possess groups which produce colour with iodine.

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STUDIES IN THE FLUORESCENCE OF DYESTUFFS.

By Sunil Bihari Sen-Gupta.

The fluorescence and extinction coefficients of a number of dyestuffs in alcoholic solutions have been studied

The König Marten spectrophotometer, which has been used in measuring absorption and fluorescence, admits of an easy separation of sodium lines 5890Å/5896Å. It has been found that absorption capacity per molecule increases with dilution up to a certain limit after which Beer's law is obeyed. The absorption in ultraviolet has also been studied.

For the determination of maximum fluorescence yield of dyestuffs and for its variation with concentrations, three different absolute photometric methods have been followed by observing the fluorescence light (*a*) opposite to, (*b*) in the same as, and (*c*) normal to the direction of exciting light. The maximum fluorescence yield for fluorescein at 365 $\mu\mu$, is about 0·72. The experimental results confirm the general observation that the fluorescence yields remain constant up to a limiting concentration, beyond which the yields fall off with increase in concentrations. Wawilow's method of determining fluorescence yields by comparing the relative intensities at a particular wave-length at different concentrations of a particular dyestuff gave some time, incorrect results and the absolute photometric method has been advocated for accurate results.

The influence of wave-lengths of exciting light on the fluorescence yields, has been studied and the yields have been found to be roughly proportional to the wave-lengths of exciting light.

The molecular conductivities of some of the purified dyestuffs in aqueous alcohol have been determined. The molecular conductivities when plotted against square root of concentration, do not give linear relationship even at 0·01*M* as is to be expected from Debye-Hückels' theory.

The various mechanisms for quenching of fluorescence have been discussed and the whole experimental results tend to support the view that in solution the dyestuffs form micelle ions which break up on dilution.

The ratio of fluorescent energy to the absorbed energy for producing fluorescence has been variously designated as fluorescence yield or fluorescing power or specific capacity for fluorescence. Wood and Dunoyer ("La structure de la Matiere", 1921, p. 307) have shown that for resonance radiation in vapours of sodium and mercury, the fluorescence yield is unity. In the case of many dyestuffs in solution Wawilow (*Z. Physik*, 1924, **22**, 266) found the ratio to be much less than unity.

Szczeniowski (*Bull. Int. Acad. Polonaise*, 1927, **A**, 127) studied the aqueous solution of sodium fluoresceinate with the aid of a photoelectric cell and found this ratio to be 0·71.

The solution of dyestuffs may be excited to emit fluorescence by light of all wave-lengths shorter than the wave-lengths of fluorescence band (Stoke). The determination of relation between the specific exciting power, *i.e.*, the fluorescence excited per unit of absorbed radiation and the wave-lengths of exciting light is a problem of considerable interest.

Einstein postulated on the basis of quantum theory that the velocity of photochemical reaction should be inversely proportional to frequency of exciting light. If fluorescence be produced by absorption in quanta, the fluorescence yield should depend on the wave-lengths of the exciting light. Nichols and Merrit (*Phys. Rev.*, 1910, **31**, 381) noted a decrease in energy efficiencies of eosin and resorufin at shorter wave-lengths and this decrease appeared to be of the same order of magnitude as one would expect to find if a uniform quantum efficiency existed. Wawilow (*Phil. Mag.*, 1922, **43**, 307 ; *Z. Physik*, 1927, **42**, 311) found that in case of fluorescein, the fluorescent yield showed a definite proportionality of energy efficiencies with the wave-lengths in the region 4047Å – 2537Å. indicating a uniform quantum efficiency. In recent years Lewschin (*J. Chem. Phys. Russ*, 1931, **2**, 641), Harrison and Leighton (*Phys. Rev.*, 1931, **38**, 899) and Fabrikant (*Phys. Zeit. Sowjet Union*, 1933, *vi*, **3**, 567) showed that the efficiency is proportional to wave-lengths of exciting light under suitable conditions of experiments.

Walter (*Wied. Ann.*, 1888, **34**, 22) observed that the specific capacity for fluorescence of solutions of fluorescein remained constant with the increase in concentrations up to a certain limit and then rapidly fell off. This critical concentration according to Walter is 3×10^{-4} g. per c.c., but according to Lepine (*Ann. phys.*, 1915, **4**, 207) and Macklenbergh and Valentiner (*Z. Physik*, 1914, **15**, 267) is of the order of 10^{-5} g. per c.c. According to Perrin (*Compt. rend.*, 1924, **178**, 1978) the fluorescing power of solutions of dyestuffs decreases exponentially with increase in concentration and is given by following equation,

$$\Phi = \Phi_0 e^{-kc}$$

where Φ is the fluorescing power at concentration c and Φ_0 is the maximum fluorescing power at zero concentration.

Wawilow (*Z. Physik*, 1925, **31**, 750), however, found that his extensive experimental results could best be correlated by a modified equation of the form,

$$\Phi = \Phi_0 e^{-(kc - c_0)}.$$

Bouchard (*J. chim. phys.*, 1936, **33**, 50) has recently drawn the conclusion that the intensity of fluorescent light at concentrations, where the fluorescing power remains constant, is so feeble that the rigorous testing of Perrin's or Wawilow's equation is not possible.

The quenching of fluorescence with increase in concentrations has been variously explained by

(*a*) process of resonant induction (F. Perrin, *Ann. phys.*, 1929, **12**, 169; 1932, **17**, 283; *Compt. rend.*, 1924, **178**, 1978, 2252; J. Perrin, *Compt. rend.*, 1923, **177**, 469, 612, 665; 1927, **184**, 1097);

(*b*) deactivating collision of second kind (Wawilow, *Z. Physik*, 1925, **31**, 750);

(*c*) formation of complex micelles (Lewschin, *Z. Physik*, 1927, **43**, 230; Banow, *ibid.*, 1929, **58**, 811);

(*d*) change in electrolytic dissociation (Sherill, Desha, and Harrison, *J. Amer. Chem. Soc.*, 1926, **48**, 1493; Eisenbrand, *Z. physikal. Chem.*, 1929, **144A**, 441; Bouchard (*loc. cit.*).

The absorption spectra of fluorescent dyes in solution have been studied in recent years by Banow (*Z. Physik*, 1929, **58**, 811; 1930, **64**, 121). Speas (*Phys. Rev.*, 1928, **31**, 569), and Lewschin (*loc. cit*). Banow has shown that the changes in absorption bands with increase in concentrations are similar in nature to those observed during the process of adsorption of dyestuffs on colloidal particles. Lewschin found that change in absorption spectra was marked at a concentration where quenching of fluorescence begins.

The subject of the molecular conductivity and transport number of ions in solutions of electrolytes which can gave rise to micelles, is now engaging considerable interest (Robinson and Moillet, *Proc. Roy. Soc.*, 1934, **A143**, 630; Lottermoser and Puschell, *Kolloid Z.*, 1933, **53**, 175; Hartley, Collie and Samis, *Trans. Faraday Soc.*, 1936, **32** 795). Many dyes fall within this class and Robinson (*Trans. Faraday Soc.*, 1935, **31**, 245), has recently shown that methylene blue, which has been uptil now regarded as a dyestuff and which forms a true solution, gives a maximum in the value of molecular conductivity with increase in concentrations indicating the formation of ionic micelles.

In this investigation, we have studied simultaneously the fluorescing power under different conditions of excitation, the absorption spectra and the molecular conductivity of certain dyes in alcoholic solution and the changes that are observed in these magnitudes as the concentration of the solution increases and an attempt has been made to correlate these properties in the interpretation of our experimental results.

EXPERIMENTAL.

The dyestuffs were very carefully purified before use. The salting out process of Robinson(*Proc. Roy. Soc.*, 1931, **A131**, 576, 596) was not found to be satisfactory. Dyestuffs were purified by crystallisation from suitable solvents. Fluorescein was purified by repeated crystallisation from acetic acid ; eosin, rhodamine B from acetone ; acriflavine, benzoflavine, rhodulin orange and rhodamine 6 G from aqueous alcohol; magdala red, phosphine 2 G and phloxine from alcohol. The concentrations have been expressed in gram molecules per litre, the molecular formulæ being as given in Colour Index (edited by F. M. Rowe, 1st Ed.).

1.	Fluorescein ...	$C_{20}H_{10}O_5Na_2$	6.	Rhodamine B ...	$C_{28}H_{31}N_2O_3Cl$.
2.	Eosin ...	$C_{20}H_6O_5Br_4Na_2$	7	Acriflavine ...	$C_{14}H_{14}N_3Cl$.
3.	Rhodamine 6 G ...	$C_{28}H_{27}N_2O_3Cl$	8.	Benzoflavine ...	$C_{21}H_{20}N_3Cl$.
4.	Rhodulin orange ...	$C_{17}H_{20}N_3Cl$.	9	Phloxine ...	$C_{20}H_4O_5Cl_2Br_4K_2$.
5.	Magdala red ...	$C_{30}H_{20}N_3Cl$.	10.	Phosphine 2 G ...	$C_{20}H_{18}N_3NO_3$.

Absolute alcohol was used throughout in making solution of these dyes. For measurements of conductivity, aqueous alcohol of specific conductance of 3×10^{-7} reciprocal ohms at 29°5 was used to have reproducible and accurate readings.

Absorption Spectra.

The absorption spectra as well as fluorescent intensities in the visible region were measured by a König-Marten spectrophotometer which was specially constructed for the purpose by Schmidt and Haensch. It contained a prism of very high dispersion, enabling an easy separation of the sodium lines 5820Å and 5896Å. The wave-lengths were calibrated on a drum by the rotation of which any monochromatic radiation could be separated. The molar extinction coefficients ϵ were calculated from the equation

$$\epsilon = \frac{\log_{10} \tan \theta_1 - \log_{10} \tan \theta_2}{c.d}$$

where d is the thickness of the cell in centimeters, and θ_1 and θ_2 are the angles for equal illumination with solutions and pure solvents respectively. Rectangular cells made of plane parallel glass plates with a stopper at the top, were used and their thickness varied between 0·025 to 10 cm. in order to avoid errors arising from possible failures of Beer's law.

For the measurements of extinction coefficients in the ultraviolet, the Adam Hilger outfit with a sector photometer and a quartz spectrograph was used. These results were further verified by the measurements of absorption with a sensitive Moll thermopile and galvanometer system. A parallel beam of rays from a point source quartz-mercury lamp was allowed to fall on a cell containing dye solution through a combination of filters, Schott and Gen UG2, 1 mm. thick and BG 4, 2 mm. thick, which allow only 365 $\mu\mu$ to pass. The incident and transmitted radiations were measured with Moll thermopile and galvanometer. The extinction coefficients, ϵ, were calculated from the equation

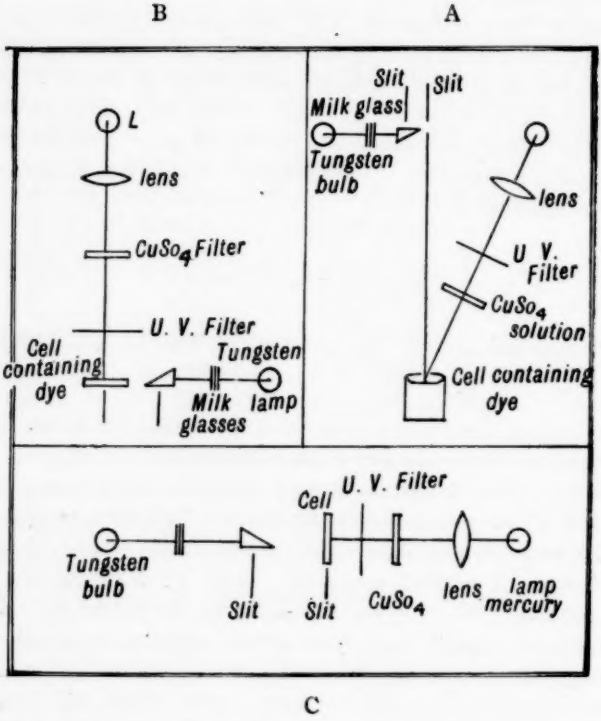
$$I_{\text{transmitted}} = I_{\text{incident}} \cdot 10^{-\epsilon c.d}.$$

Quantitative Methods of Measurements of Fluorescence Yields by Absolute Photometric Methods.

Three methods have been followed in this investigation for the quantitative measurements of fluorescence yields of dyestuffs.

Method A.—For observation of fluorescence in concentrated solutions ($M/1000$ and above) and for noting the effect of different thicknesses of cell on critical concentrations beyond which the fluorescence yield diminishes, the spectral photometric method, as usually adopted by Wawilow (*Z. Physik*, 1924, **22**, 266; 1925, **31**, 750), was followed. In this method the fluorescent light was observed opposite to the direction of incidence of exciting light. The exciting light was a point-o lite quartz-mercury lamp, placed in a metallic jacket with a window opposite to point source. This light was made parallel by use of a quartz lens, placed suitably in a metallic jacket. The parallel beam of light was allowed to fall upon the surface of the cell containing dye solution, through an ultraviolet filter (Schott and Gen U G 2, 1 mm. thick) and of 1 cm. thick cell containing very dilute solution of copper sulphate. The rectangular cells containing the fluorescent solution or the filtering solution were made of plane parallel plates of fused quartz with a stopper at the top. The mercury-quartz lamp was placed on one side of the König-Marten spectrophotometer and the angle at which the light fell upon the surface never exceeded 20°. The quartz-mercury lamp was run at a constant current of 2 amperes, fed from a storage battery of 30 volts. The fluorescent light was compared with the light from a tungsten lamp. The comparison tungsten lamp was enclosed in a tubular metallic jacket and light from this source entered one of the slits of König-Marten spectrophotometer through a right angled prism, while the fluorescent light entered the other

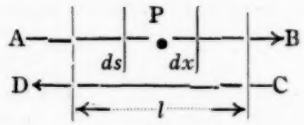
FIG. 1.



slit. The intensity of light from the tungsten lamp could be varied at will by placing milk glasses in front of the lamp. The tungsten lamp was run from a storage battery of 4 volts and its energy of radiation at various wavelengths was calibrated with the aid of a standard lamp supplied by Kipp and Zonen. The whole experimental arrangement can better be understood from the diagram (Fig. 1A).

With this mode of excitation, the flouorescence yields can be calculated as follows:

Let us suppose for sake of simplicity, that the exciting light is falling in the direction of AB and the emitted light is observed along the reverse direction CD, then the contribution to the intensity of fluorescent light in a solid angle dw from any volume $ds.dx$ round a point P within the cell will be



$$dF_{\lambda_1} d\lambda_1 = KCI_\lambda \; d\lambda \; a_{\lambda_1} \; d\lambda_1 \; ds \; e^{-k.c.x} \; dx . e^{-k'cx} \; \frac{dw}{4\pi}$$

where $I_\lambda \; d\lambda$ is the energy of incident light expressed in ergs/cm.^{2}/sec.

$a_{\lambda_1} \; d\lambda_1$ is the fraction of the absorbed energy transformed into fluorescent energy in the spectral region λ_1 and $\lambda_1 + d\lambda_1$.

K is the molar extinction coefficient of solution for exciting light (wavelength λ) ;

K', the molar extinction coefficient of solution for fluorescent light (wavelength λ_1) ;

C, the molar concentration of dyestuff solution and,

ds is the effective area which fills up the solid angle opening dw of the slit.

On integrating, the above equation within the limit o and 1, the thickness of the cell, we shall get the total fluorescent light $F_{\lambda_1} \; d\lambda_1$ emerging from the cell in a solid angle dw from the surface ds.

$$F_{\lambda_1} d\lambda_1 = I_\lambda \; d\lambda \; ds . \frac{dw}{4\pi} . K.C. \; a_{\lambda_1} \; d\lambda_1 \int_0^1 e^{-(k+k')c.l} \; .dx$$

$$= I_\lambda \; d\lambda ds . \; \frac{dw}{4\pi} . \frac{KC}{(k+k') \; C} . \left[1 - e^{-(k+k') \; c.l} \right] a_{\lambda_1} d\lambda_1 .$$

Now, $dw = \frac{\triangle\sigma}{R^2}$, where $\triangle\sigma$ is the area of the slit opening and R is the distance of the cell to the slit, *i.e.*

$$F_{\lambda_1} \; d\lambda_1 = \frac{I_\lambda \; d\lambda}{4\pi} . \triangle\sigma \; \frac{ds}{R^2} . \frac{K}{(k+k')} \left[1 - e^{-(k+k') \; c.l} \right] a_{\lambda_1} d\lambda_1 .$$

If the arrangement is such that the fluorescent light fills completely the aperture of the spectrophotometer, the total surface of the cell from which the fluorescent radiation is received may be regarded as $= A \cdot R^2 / f^2$, where A/f^2 is the effective aperture of the instrument (A, the area and f, the focal

length of the lens), taking this into account, the expression becomes

$$F_{\lambda_1} d\lambda_1 = \frac{I_\lambda \, d\lambda}{4\pi} \cdot \triangle\sigma \frac{A}{f^2} \cdot \frac{K}{k+k'} \left[1 - e^{-(k+k')\, c.l} \right] a_{\lambda_1} d\lambda_1$$

thus the intensity observed will be independent of the distance of the fluorescent cell.

We made some preliminary experiments to test this and found that the intensity of fluorescent light remains practically independent of the distance of the cell from the slit provided that the portion illuminated, may fill up the effective aperture.

Now the fluorescent light $F_{\lambda_1} d\lambda_1$ was compared with the radiation from a comparison lamp $I_{0\lambda_1} d\lambda_1$ coming from a uniform source transmitted through milk glasses. This light (standardised by Kipp and Zonen band lamp) entered the same slit (divided into two by a diaphragm) having the same slit area and filled up by the same solid angle A/f^2.

The effective intensity of radiation from the comparison lamp between wave-lengths λ_1 and $\lambda_1 + d\lambda_1$ at the slit would be

$$I_{\lambda_1} d\lambda_1 = I_{\lambda_1} d\lambda_1 . \Delta\sigma . \frac{A}{f^2} .$$

Now, we compare the intensities of these two radiations by means of the same spectrophotometer by rotating the nicol and finding out the angles for equal illumination; in this case we have,

$$F_{\lambda_1} d\lambda = I_{\lambda_1} d\lambda_1 \frac{1}{\tan^2 \theta} ,$$

where θ is the angle for equali llumination, taking the zero reading to be at 45°. Then

$$\frac{F_{\lambda_1} d\lambda_1}{I_{\lambda_1} d\lambda_1} = \frac{I_\lambda \, d\lambda}{I_{0\lambda_1} d\lambda_1} \cdot \frac{K}{4\pi (k+k')} \cdot \left[1 - e^{-(k+k')\, c.l} \right] a_{\lambda_1} d\lambda_1$$

$$= \frac{I}{\tan^2 \theta} \quad \text{or}$$

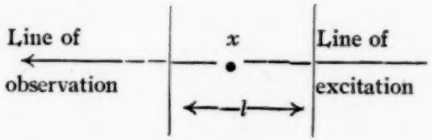
$$\frac{I}{\tan^2\theta} = I_\lambda\, d\lambda\, \frac{K}{4\pi(k+k')}\left[1-e^{-(k+k')\,c.l}\right]\frac{a_{\lambda_1}\, d\lambda_1}{I_{0\lambda_1}\, d\lambda_1}.$$

In this equation, the values of all the terms are known and hence the value of $a_{\lambda_1} d\lambda_1$ can be calculated for each wave-length of the fluorescence spectrum at each concentration of dyestuffs. These values are plotted in the graph and the integrated area would be equal to $I_{\lambda_1} d\lambda a$. The value of $I_\lambda\, d\lambda$, the total incident light in ergs/cm.^{2}/sec. is known, the corresponding value of 'a' for each concentration is, thus, known.

Method B.—This method of observing fluorescent light in the direction of incident exciting light is very convenient for the study of the fluorescence in dilute solution ($M/1000$ and below). The experimental arrangement was very simple and may easily be understood from the diagram (Fig. 1B). A quartz lens system of 2·5 cm. focal length was used to have a strong beam of parallel rays from the point source quartz-mercury lamp. The filtering device for obtaining monochromatic radiation and the arrangement for the comparison of intensities of light are the same as in method A.

Since there are few corrections which are to be made when the exciting source is monochromatic, extensive measurements of fluorescence were made with the ultraviolet radiation ($365\mu\mu$) from mercury-quartz lamp as the source of excitation. This mode of excitation has the advantage that scattered light from the cell would not affect our results and our data will be more dependable than those of the previous investigators in this field.

With this method, the calculation of fluorescence yield was made as follows:—



The intensity of fluorescent light at any point in the cell of thickness 1, will be

$$dF_{\lambda_1} = \frac{I_\lambda d\lambda a_{\lambda_1}\, d\lambda_1}{4\pi}.e^{-kx}\, K.dx\, e^{-k'(1-x)}.$$

Other factors, which ultimately vanish and do not come into consideration, are not put here for the sake of simplicity of mathematical expression The symbols have the usual significance as described in Method A.

Total fluorescent light emerging from the cell will be

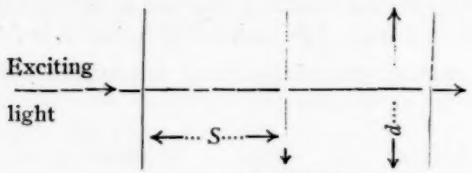
$$F_{\lambda_1}\, d\lambda_1 = \frac{I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1}{4\pi} . K.e^{-kl} \int_0^1 e^{-(k-k')x} .dx$$

$$= \frac{I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1}{4\pi} \cdot \frac{K}{k-k'} . e^{-k'l} \left[1 - e^{-(k-k')l} \right]$$

The factor $\frac{F_{\lambda_1}\, d\lambda_1 4\pi(k-k')}{K.e^{-k'l}\ [\ 1-e^{-(k-k')l}\ ]}$ can easily be calculated for each wave-length of fluorescent light. By plotting these values for each concentration, we should get the integrated area as equivalent to $I_\lambda\, d\lambda a$ for that concentration. $I_\lambda\, d\lambda$ being known, 'a' for each concentration can easily be calculated.

Method C.—The above two methods are not suitable for the study of the effect of the wave-lengths of exciting light on fluorescence yields of dyestuffs, because the scattered rays from visible exciting source would affect our results. For the study of the effect of wave-lengths of exciting light the following method of observing fluorescent light normal to the direction of exciting light was adopted. (Fig. 1*c*).

With this mode of excitation, the fluorescent light $F_{\lambda_1} d\lambda_1$, would be



$$F_{\lambda_1}\, d\lambda_1 = \frac{I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1}{4\pi} \cdot \frac{k}{k'} . e^{-ks} \left[1 - e^{-k'd} \right]$$

where d is the thickness of the cell, S, the distance of the surface layer of the solution to the layer along whole of which the fluorescent light is falling on the slit of the spectrophotometer. Other symbols have the usual significance (Method A).

For higher values of $k'd$ (at shorter wave-length)

$$F_{\lambda_1}\, d\lambda_1 = \frac{I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1}{4\pi} \cdot \frac{K}{k} . e^{-ks}$$

and for smaller values of $k'd$ (*i.e.* at longer wave-lengths)

$$F_{\lambda_1} d\lambda_1 = \frac{I_\lambda \; d\lambda a_{\lambda_1} \; d\lambda_1}{4\pi} \; . K.d. \; e^{-ks}.$$

In these equations, all factors are known, 'a' can be calculated as mentioned before.

With this mode of excitation, a small amount of scattered and diffusedly reflected light was unavoidable, the correction to be applied on this account was determined by making measurements throughout the spectrum, when the cell was filled up with solvent alone, everything else remaining same.

Isolation of Monochromatic Light for Excitation.

Most of the investigators employed a monochromator to study the influence of each wave-length on the intensity of fluorescent light. The exciting light when passed through a monochromator is of so feeble intensity that accurate study of the problem is not possible. Moreover, the selection of wave-lengths at short intervals, is of little value because the variation would be so small that it may be counted as experimental errors. In view of all these difficulties, we have employed in the present investigation, the following suitable monochromatic filters to isolate the mercury lines.

313 $\mu\mu$—Chance Bros. U. V. filters 1 mm. thick+1 cm. of 5% $CuSO_4$ solution +1 cm. 0·02% $K_2Cr_2O_7$ solution.

365 $\mu\mu$—Schot and Gen filter U. G. 2, 1 mm. thick+B.G. 4, 2 mm. thick.

404 $\mu\mu$—Wratten violet filter+1 cm. of 2% $CuSO_4$ solution.

436 $\mu\mu$—Zeiss monochromatic filter C+1 cm. of 1% $CuSO_4$ solution.

546 $\mu\mu$—Zeiss monochromatic filter B+1 cm. of 5% $CuSO_4$ solution.

577 $\mu\mu$, 579 —Zeiss monochromatic filter A+1 cm. of 5% $CuSO_4$ solution.

Calibration of Comparison Lamp.

The band lamp No. 205, used in this investigation, has been calibrated at Physical Laboratory of the University of Utrecht, Holland. The calibration method was described by L. S. Ornstein, D. Vermulen, and E.F M. V.d Held (*J. Opt. Soc. Am.*, 1930, **20**, 573). The use of this lamp in

spectroscopic work has been described by Orstein, Moll and Burger in their book " Objektive spectral Photometrie " (Vieweg, Braunschweig).

The absolute energy in ergs per Ångstrom per sec. per unit solid angle at wave-length 6000Å at different current intensity is given in a tabular form. The certificate of calibration supplies the colour temperature of the band lamp as a function of current intensity through the lamp. A table containing the values of $E_\lambda / E_{max.}$ as a function of λT, where T is colour temperature, was also supplied. True temperature corresponding to colour temperature is found out from a table. Thus the relative distribution of energy for other wave-lengths can be calculated and in doing so, a slight correction due to spectral emissivity of tungsten at different wave-lengths is also considered.

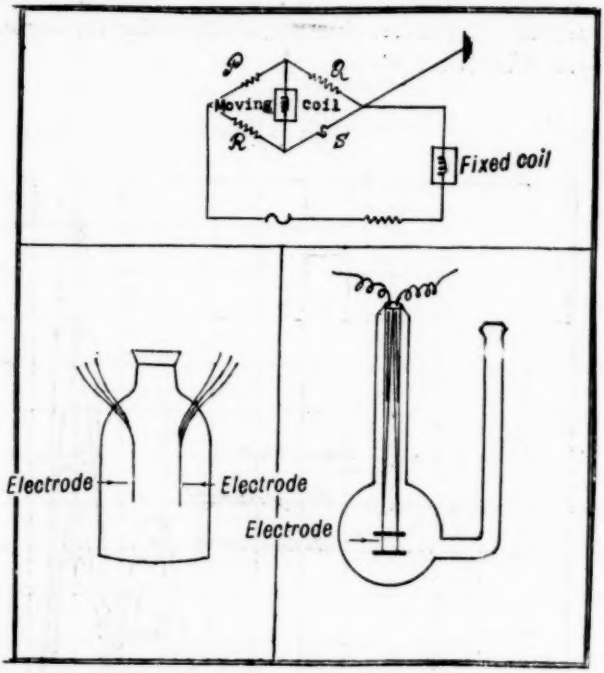
Measurements of Molecular Conductivities of Dyestuffs in Alcoholic Solution.

All the measurements were done in dilute solutions, where correction due to the conductivity of alcohol has a great significance.

All the measurements were made in a water thermostat at the temperature specified in each case. The temperature was kept accurate and constant within $\pm 0.005°$ by use of an electric thermo-regulator.

Apparatus.—The conductivities were measured with the help of an alternating current galvanometer of Wattmeter type devised by H. Mukherjee (*Z. Physik*, 1930, **64**, 286). The galvanometer gave accurate and reproducible readings at 30-50 milliamperes. The sensitiveness of the galvanometer was about 2×10^{-7} amperes, when the current in the fixed coil was 50 milliampere. The experimental errors did not exceed 0·1%, even when the highest resistance was measured. The resistance boxes were all non-inductively wound variable resistances from 0·1 to 10,000 ohms. The current was supplied from a double contact tuning fork oscillator which was found to give equally intense positive and negative current by means of a oscillograph. The current from the oscillator was made to pass through a variable resistance box, by the adjustment of which, current intensities could be changed. The arrangement for measurements will be clear from the diagram (Fig. 2). Before working, it is necessary that galvanometer should remain in electrically symmetrical position between the fixed coils. This was assured by passing a current of 50 milliamperes through the fixed coils and short circuiting the moving coils and adjusting the position of the latter when there was no current. A tapping key was placed in the battery circuit so that no current was passing through the solution except in time of taking readings.

FIG. 2.



A B

Two types of cells made of Jena glass used in covering the range of conductivity from 500×10^{-6} to 1×10^{-6} reciprocal ohms are shown in the diagram (Fig. 2).

The cell 'A', which was used for solutions of relatively high conductivities, had a cell constant 0·148, a capacity of 80 c. c. and rectangular smooth platinum electrodes. The cell B had a cell constant 0·04517, a capacity of 80 c. c. was used in measuring low conductivities. The electrodes were stout and smooth sheet of platinum whose relative positions were fixed by sealing with glass. Smooth platinum was used as the dyestuffs were found to be adsorbed on black or grey platinum and reproducible readings could not be obtained. Readings were taken at two different frequencies of oscillator.

Results and Discussion.

Absorption Spectra of Alcoholic Solutions of Dyestuffs.

In the following pages the molecular extinction coefficients of dyestuffs in two or three concentrations are given graphically against the wave-lengths expressed in $\mu\mu$ (Figs. 3, 4, 5, 6).

Fig. 3.

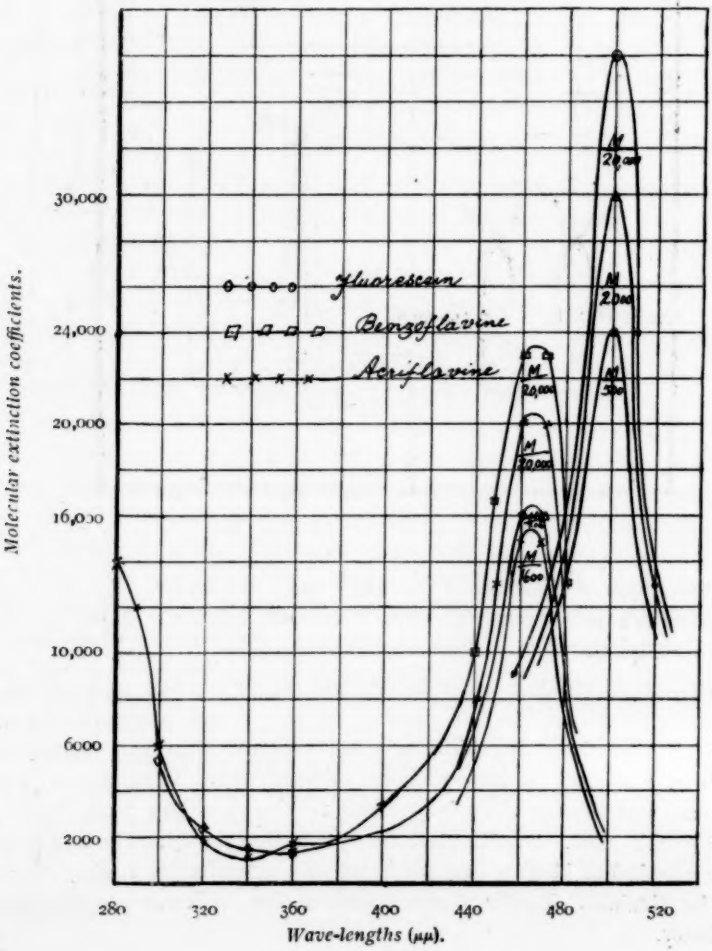
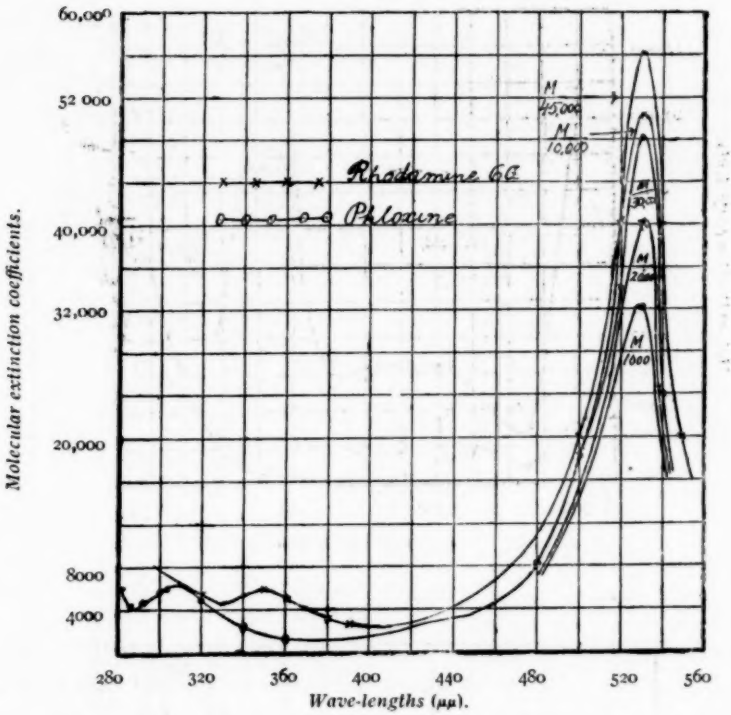


FIG. 4.



We have observed in all cases excepting for rhodamine B and phosphine-2 G that the molecular absorption coefficients increase with dilution up to a certain limit after which Beer's law is obeyed. This means that the ratio of the number of molecules with greater absorbing power to the total number of molecules increases with decrease in concentration of dyestuffs This may be possible if molecular aggregation takes place at concentrated solutions and aggregates have less absorbing power than the molecules from which they are formed ; dilution, favouring the breaking up of micelles, increases the relative number of simple molecules. We have not observed any change in absorption curves with increase of concentrations as Söderborg (*Ann. Physik*, 1913, **41**, 381), and Van der Plaats (*ibid.*, 1915, **47**, 429) observed in case of aqueous solution. This we may explain, by assuming that in alcoholic solution a slight aggregation takes place with increase of concentration, but in aqueous solution the dye molecules suffer considerable aggregations with increase of concentrations. This has been confirmed by

FIG. 5.

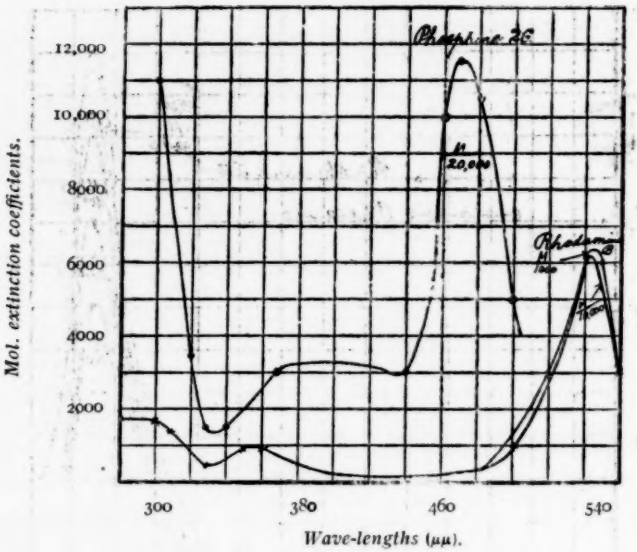

Phosphine 2G
M/20,000
Rhodamine B
M/100
M/1000
12,000
10,000
8000
6000
4000
2000
300
380
460
540
Mol. extinction coefficients.
Wave-lengths (μμ).

FIG. 6.

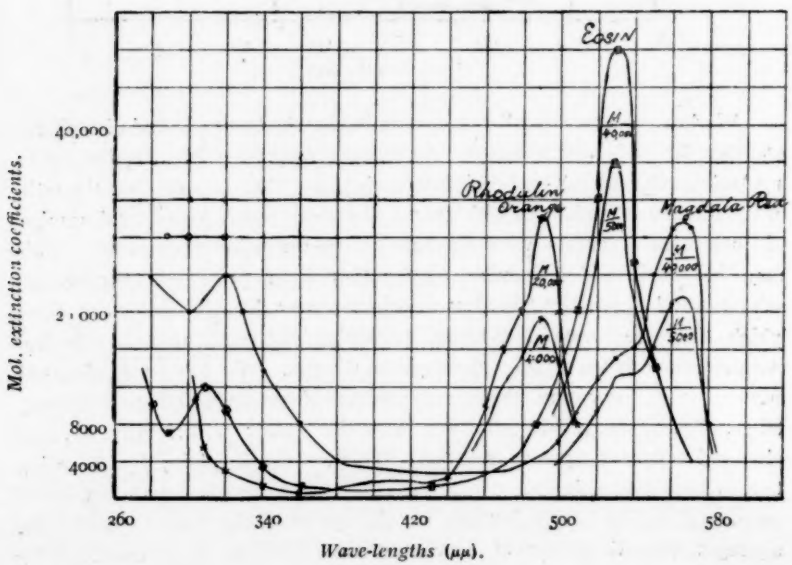

Eosin
M/40,000
M/5000
Rhodulin Orange
M/20,000
M/40,000
Magdala Red
M/40,000
M/5000
40,000
2 1 000
8000
4000
260
340
420
500
580
Mol. extinction coefficients.
Wave-lengths (μμ).

FIG. 7.
Acriflavine.

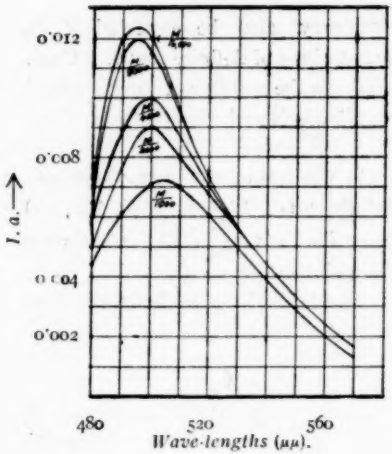


FIG. 8.
Acriflavine.

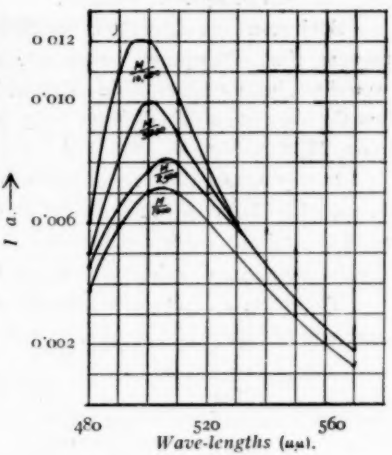


FIG. 9.
Rhodamine 6 G.

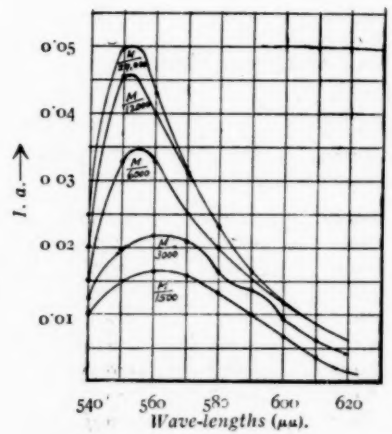
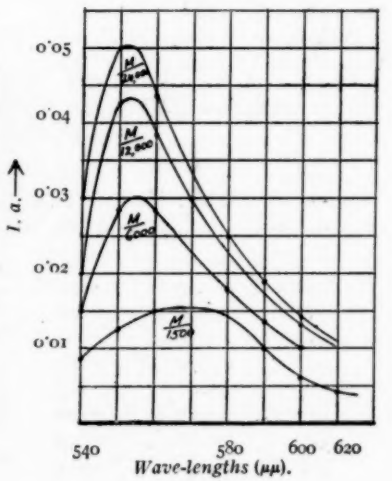


FIG. 10.
Rhodamine 6 G.



Söderborg (*loc. cit.*) and Banow (*Z. Physik*, 1929, **58**, 811), who have shown that a rapid extinction of fluorescence with a considerable and a relatively slow extinction of fluorescence with a slight alteration of absorption spectra. This has also been further confirmed by Mlle Vitte (*J. chim. phys.*, 1926, **23**, 276), Bouchard (*ibid.*, 1936, **33**, 127), who observed that decrease o

fluorescing power of dyestuffs with concentrations is less rapid in alcoholic solution than in aqueous solution.

Such results in absorption coefficients may also be expected if we assume that absorption bands are due to ions of definite type. There would not be any appreciable shift of band as the number of ions is varied but the absorption coefficients may vary (Nichols and Merrit, *Phys. Rev.*, 1904, **19**, 18; 1910, **31**, 376).

It may be added here that our results in absorption in ultraviolet may be useful in the sense that recently Grisebach (*Z. Physik*, 1936, **101**, 13) tried to establish a relation between absorption curves with polarisation curves specially as to the cause of negative polarisation.

The molar extinction coefficients obtained by both the methods at $365\mu\mu$ gave almost concordant results, as will be evident from Table I.

TABLE I.

Dyestuffs.	Mol. extinct. coeff. at 365 $\mu\mu$ by Thermoelectric method.	Photographic method.	Dyestuffs.	Mol. extinct. coeff. at 365 $\mu\mu$ by Thermoelectric method.	Photographic method.
Fluorescein	2000	2100	Magdala red	7800	7400
Eosin	1200	1200	Benzoflavine	1100	1200
Rhodamine 6 G	3600	4000	Acriflavine	2000	2000
Rhodulin orange	600	600	Phosphine 2G	2300	2500
Rhodamine B	550	625	Phloxine	1200	1200

Influence of Thickness of Cell on Fluorescence Yields.

TABLE II (*vide* Fig. 7).

Acriflavine.

Cell—5 mm. thick. Method of excitation—A. $I_\lambda\, d\lambda = 16$ ergs/cm.^{2}/sec.

Wave-lengths.	$I_\lambda d\cdot a_{\lambda_1} d\lambda_1$ for concentrations of				
	M/1000.	M/2000.	M/4000.	M/8000.	M/16,000
480 $\mu\mu$	0·0044	0·005	0·006	0·0065	0·007
490	0·0063	0·0077	0·009	0·0115	0·012
500	0·007	0·009	0·01	0·0115	0·012
510	0·0072	0·0085	0·009	0·01	0·01
520	0·006	0·0065	0·007	0·0074	0·0074
530	0·005	0·0055	0·0058	0·0058	0·0058
540	0·004	0·0045	0·0045	0·0045	0·0045
550	0·003	0·0035	0·0035	0·0035	0·0035
560	0·002	0·0025	0·0025	0·0025	0·0025
570	0·0012	0·0017	0·0017	0·0017	0·0017

$I_\lambda d\lambda = 16$ erg /cm.^{2}/sec.

Conc. ...	M/1000	M/2000	M/4000	M/8000	M/16000
$I_\lambda d\lambda$ ...	4·2	5·06	5·6	6·1	6·4
a ...	0·26	0·316	0·35	0·38	0·40

TABLE III (*vide* Fig. 8)

Acriflavine.

Cell—20 mm. thick.

Wave-lengths.	$I_\lambda d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of M/1000.	M/2500.	M/5000.	M/10,000.	M/20,000.
480 μμ	0·004	0·0047	0·005	0·055	0·0062
490	0·006	0·0065	0·0076	0·01	0·01
500	0·007	0·0076	0·01	0·012	0.012
510	0·0072	0·0080	0·009	0·01	0·01
520	0·006	0·0065	0·007	0·0078	0·0078
530	0.005	0·0055	0·0057	0·0061	0·0061
540	0·004	0.0045	0·005	0·0055	0·0055
550	0·0025	0·003	0·0035	0·0038	0·0038
560	0·0020	0·0022	0·0025	0·00028	0·0028
570	0·0012	0·0015	0·0018	0·002	0·0020

$I_\lambda \ d\lambda = 16$ ergs/cm^2./sec.

Conc. ...	M/1000	M/2500	M/5000	M/10,000	M/20,000
$I_\lambda \ d\lambda \ a$ ...	4·20	4·45	5·62	6·30	6·5
a ...	0·26	0·28	0·35	0·394	0·405

TABLE IV (*vide* Fig. 9).

Rhodamine 6 G.

Cell—5 mm. thick.

Wave-lengths.	$I_\lambda d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of M/1500·	M/3000.	M/6000.	M/12,000.	M/24,000
540 μμ	0·01	0·012	0·016	0·02	0·025
550	0·015	0·02	0·033	0·045	0·05
560	0·016	0·023	0·033	0·04	0·042
570	0·016	0·021	0·025	0·03	0·03
580	0·013	0·016	0·0197	0·021	0·02
590	0·01	0·0125	0·0158	0·0155	0·0155
600	0·0064	0·008	0·01	0·01	0·01
610	0·004	0·006	0·0075	0·008	0·008

$I_\lambda \ d\lambda = 25$ ergs./cm^2./sec.

Conc. ...	M/1500	M/3000	M/6000	M/12,000	M/24,000
$I_\lambda d\lambda a$ ...	8·8	12·05	15·65	18·25	19·25
a ...	0·352	0·48	0·626	0·73	0·77

TABLE V (*vide* Fig. 10).

Rhodamine 6 G.

Cell—20 mm. thick.

Wave-lengths.	$I_\lambda d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of				
	M/1500.	*M*/6000.	*M*/12,000.	*M*/24,000.	*M*/48,000.
540μμ	0·01	0·015	0·02	0·03	0·03
550	0·0125	0·0288	0·043	0·05	0·05
560	0·015	0·028	0·037	0·042	0·042
570	0·0155	0·02	0·025	0·03	0·03
580	0·015	0·018	0·02	0·022	0·022
590	0·01	0·012	0·015	0·017	0·017
600	0·006	0·01	0·012	0·015	0·015
610	0·004	0·006	0·008	0·012	0·012

$I_\lambda\, d\lambda = 25$ ergs./cm².sec.

Conc. ...	*M*/1500	*M*/6000	*M*/12000	*M*/24000	*M*/48000
$I_\lambda d\lambda a$ ...	8·05	13·05	17·05	19·5	19·5
a ...	0·32	0·52	0·70	0·78	0·78

It will appear from the above experimedtal results that if readings be taken on the red end of the fluorescence spectrum as Wawilow (*loc. cit.*) did, the critical concentrations obtained will be different for different thicknesses of the cell as will be evident from the following summarised data.

Cell thickness.	Concentration at which $I_\lambda d\lambda a_{\lambda_1} d\lambda_1$ remains constant	
	Acriflavine.	Rhodamine 6 G.
20 mm.	*M*/10,000	*M*/24,000
5	*M*/2000	*M*/6000

FIG. 11.

Fluorescein.

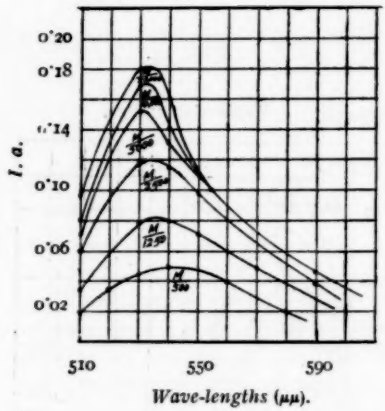
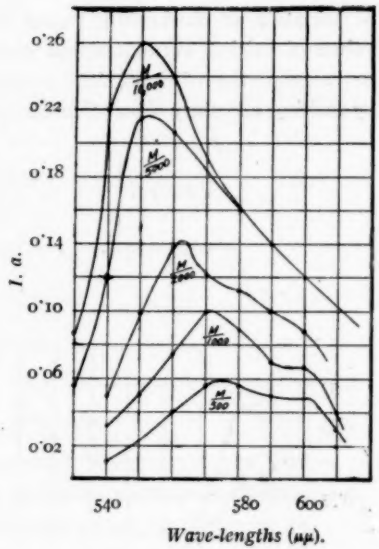


FIG. 12.

Eosin.



Such results may be explained from the previous mathematical deduction (Method A) in which we have shown

$$F_{\lambda_1} d\lambda_1 = \frac{I_\lambda d\lambda a_{\lambda_1} \, d\lambda_1 \, k}{4\pi(k - k')} \cdot \left[1 - e^{-(k+k')c.l} \right]$$

On expanding and neglecting the value of k and k', which are evidently small in dilute solution at red end of the spectrum, we get

$$F_{\lambda_1} d\lambda_1 = \frac{I_\lambda d\lambda a_{\lambda_1} \, d\lambda_1 k.c.l}{4\pi}$$

That is, the fluorescent intensity ($F_{\lambda_1} d\lambda_1$) will be proportional to concentration of dyestuffs and thickness of cell. Therefore, in dilute solution, for similar values of $F_{\lambda_1} d\lambda_1$ with increase of thickness of cell, a proportionate decrease of concentration of dyestuffs is expected, if all the experimental conditions satisfy the ideal condition, *i.e.*, if k and k' are small and absorption coefficient follows Lambert's law.

It is, therefore, clear that Wawilow's method of studying the fluorescing power by taking the fluorescent intensity readings at red end of the fluorescence spectrum may enable us only to gain knowledge about the law of variation of fluorescing power with concentrations but not about the values of critical concentrations of dyestuffs for which purpose, we are to calculate the whole of the fluorescence energy emerging from a solution by making necessary absorption corrections.

FIG. 13.

Rhodamine 6 G.

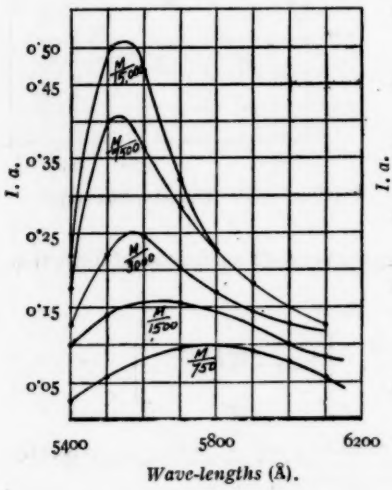
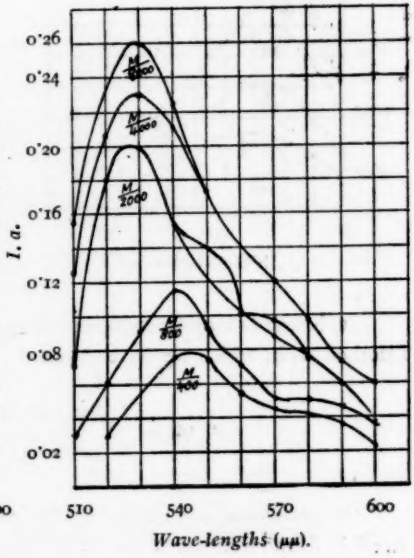


FIG. 14.

Rhodamine orange.



Influence of the Thickness of Cell on Fluorescence Yields.

It is worth while determining carefully if fluorescence yields of dyestuffs solution for the same concentrations vary with the thickness of the cell. In the following table, the values of '*a*' (the yields) for each concentration at different thicknesses of cell are given. The values are obtained from graphical interpolation (when required) of the experimental data recorded in previous tables.

TABLE VI.

Acriflavine.

Thickness of cell.	Values of 'a' at concentrations of			
	M/1000.	M/2000.	M/4000.	M/8000.
5 mm.	0·26	0·31	0·35	0·38
20 mm.	0·26	0·28	0·33	0·385

Rhodamine 6 G.

	M/1500.	M/3000.	M/6000.	M/12,000.	M/24,000
5 mm.	0·35	0·48	0·62	0·73	0·77
20 mm.	0·32	—	0·52 (?)	0·70	0·78

Thus it appears from the above data that fluorescence yields at same concentration of dyestuffs are of the same value, whatever be the thickness of the cell. It is, therefore, clear that if fluorescence yields are calculated from entire fluorescent band by making necessary absorption corrections, the yields are independent of cell thickness.

FIG. 15.

Benzoflavine.

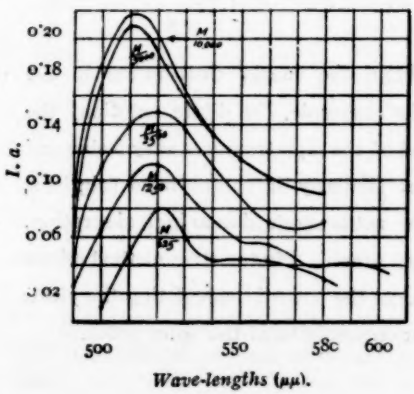


FIG. 16.

Magdala red.

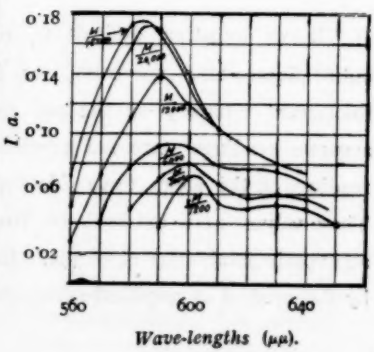


FIG. 17.

Rhodamine B.

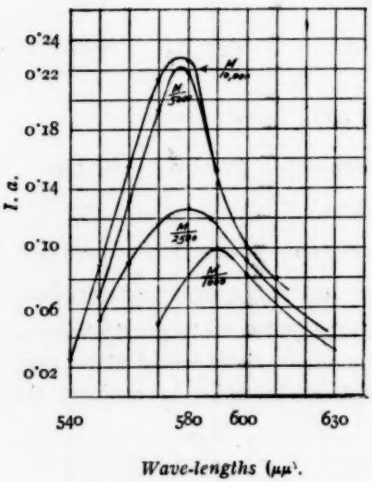
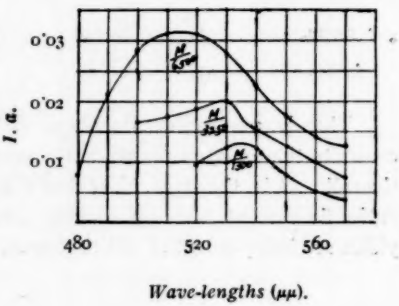


FIG. 18.

Phosphine 2 G.



Influence of Concentration of Solution on Fluorescence Yields.

It was sometime difficult to obtain the energy distribution in the entire fluorescence spectrum, as for example, in dilute solution, the fluorescent intensity on the red end of the spectrum was very feeble and accurate readings were not possible; similarly in concentrated solutions, readings on the short wave side were not accurate due to high absorption. These values were extrapolated from graph. Photometric method allows an experimental error of ± 3%. Hence the error in calculating the yields by this method of graphical extrapolation would be of the same order.

TABLE VII (*vide* Fig 11).

Fluorescein.

$p_H = 12{\cdot}0$. $I_\lambda\, d\lambda_1 = 130$ ergs/cm^2./sec.

Wave-lengths.	$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of					
	M/500.	M/1250.	M/2500.	M/5000.	M/10000.	M/20000
510$\mu\mu$	0.0255	0·038	0·06	0·07	0·08	0·081
520	0.035	0·0584	0·094	0·11	0·14	0·15
530	0·0415	0·0758	0·12	0·15	0·17	0·18
540	0·053	0·081	0·106	0·12	0·14	0·16
550	0·05	0·0707	0·094	0·096	0·11	0·11
560	0·0416	0·062	0·084	0·090	0·0955	0·091
570	0·035	0·0486	0·065	0·07	0·076	0·070
580	0·0204	0·0406	0·05	0·056	0·06	0·06
590	...	0·028	0.038	0·044	0·045	0·045
600	...	0·020	0·03	0·038	0·038	0·038

Conc. ...	M/500	M/1250	M/2500	M/5000	M/10,000	M/20,000
$I_\lambda\, d\lambda a$ ...	31·4	49·0	70·2	77·4	88·0	92 8
a ...	0·24	0·38	0·54	0·60	0·68	0·71

TABLE VIII (*vide* Fig. 12).

Eosin.

$p_H = 12{\cdot}0$. $I_\lambda\, d\lambda = 200$ ergs/cm^2./sec.

Wave-lengths.	$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of					
	M/500.	M/1000.	M/2000.	M/5000.	M/10,000.	M/20,000.
530$\mu\mu$	...	...	...	0·05	0·089	0·104
540	0·0135	0·03	0·046	0 115	0·218	0 22
550	0·02	0·053	0·10	0·215	0·26	0·28
560	0·04	0·075	0·143	0·206	0·24	0·24
570	0·056	0·10	0·122	0·18	0·18	0·18
580	0·0517	0·083	0·117	0·155	0·155	0·155
590	0·047	0 073	0·10	0·145	0·145	0·145
600	0·047	0 067	0·09	0·12	0·120	0 120
610	0 0338	0·03	...	...	...	...

Conc ...	M/500	M/1000	M/2000	M/5000	M/10,000	M/20,000
$I_\lambda d\lambda\, a$ ...	30·0	49·3	76·6	116·0	133·8	136
a ...	0 15	0·246	0·383	0·58	0·669	0·68

Remark—A subsidiary maximum at about wave-length 590-600 $\mu\mu$ is obs rved along with the principal maximum at 570 $\mu\mu$ at concentrated solutions. This maximum is shifted towards the violet end along with the principal maximum with dilution.

TABLE IX (*vide* Fig 13).

$I_\lambda\, d\lambda = 300$ ergs/cm^2/sec.

Rhodamine 6G

$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of

Wave lengths.	M/750.	M/1500.	M/3000.	M/7500.	M/15.000.	M/30,000
540$\mu\mu$	...	0·10	0·125	0·175	0·225	0·225
550	0·0306	0·125	0·22	0·40	0·50	0·50
560	0·06	0·15	0·24	0·37	0·43	0·45
570	0·0933	0·155	0·20	0·27	0·32	0·32
580	0·103	0·145	0·16	0·22	0·22	0·22
590	0·09	0·125	0·14	0·17	0·17	0·17
600	0·08	0·105	0·125	0·15	0·15	0·15
610	0·063	0·09	0·11	0·13	0·13	0·13
620	0·05	0·075	0·085	0·09	0·09	0·09

Conc. ...	M/750	M/1500	M/3000	M/7500	M/15,000	M/30 000
$I_\lambda\, d\lambda\, a$ ...	62·0	104·2	132·5	187	219	219
a ...	0·207	0·347	0·44	0·623	0·73	0·73

TABLE X (*vide* Fig. 14).

Rhodulin orange.

$I_\lambda\, d\lambda = 200$ ergs/cm^2./sec.

$I_\lambda\, d\lambda\, a_{\lambda_1}\, d\lambda_1$ at concentrations of

Wave-lengths.	M/400	M/800.	M/2000.	M/4000.	M/8000.	M/16,000.
510$\mu\mu$	...	0·0417	0·069	0·126	0·165	0·187
520	0·03	0·063	0·187	0·204	0·20	0·24
530	0·053	0·093	0·176	0·254	0·26	0·26
540	0.074	0·115	0·15	0·210	0·218	0·218
550	0·0736	0·081	0·142	0·17	0·167	0·17
560	0·053	0·072	0·10	0·143	0·142	0·145
570	0·044	0·0537	0·097	0·123	0·12	0·12
580	0·0417	0·0513	0·0714	0·10	0·10	0·10
590	0·0354	0·0478	0·057	0·066	0·066	0·066
600	0·0268	0·04	...	...	...	...

Conc. ...	M/400	M/800	M/2000	M/4000	M/8000	M/16000
$I_\lambda\, d\lambda\, a$ ...	40·72	62·02	96·31	128·0	135·0	136·0
a ...	0·2036	0·31	0·48	0·64	0·675	0·68

Remark—A slight trace of maximum is observed at about 580 $\mu\mu$ at very concentrated solutions along with the principal maximum at 545 $\mu\mu$.

TABLE XI (*vide* Fig. 15).

Benzoflavine.

$I_\lambda\, d\lambda$ = 200 ergs/cm².sec.

$I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1$ at concentrations of

Wave-lengths.	M/625.	M/1250.	M/2500	M/5000.	M/10,000.	M/20,000.
490μμ	...	0·024	0·0316	0·055	0·088	0·158
500	0·0096	0·064	0·11	0·155	0·21	0·21
510	0·046	0·10	0·14	0·21	0·20	0·20
520	0·08	0·103	0·15	0·186	0·175	0·175
530	0·06	0·10	0·13	0·158	0·162	0·162
540	0·045	0·087	0·107	0·126	0·126	0·126
550	0·043	0·056	0·09	0·114	0·114	0·114
560	0·044	0·056	0·071	0·10	0·10	0·10
570	0·043	0·0446	0·077 (?)	0·092	0·092	0·092
580	0·033	0·04	0·08 (?)	0·0867	0·0867	0·0867
Conc.	... M/625	M/1250	M/2500	M/5000	M/10,000	M/20,000
$I_\lambda\, d\lambda\, a$	... 39·1	69·2	90·3	123·3	128·1	128·0
a	... 0·196	0·346	0·45	0·616	0·64	0·64

Remark—A subsidiary maximum is observed at about 560-570μμ along with the principal maximum 520-525 μμ at concentrated solutions.

TABLE XII (*vide* Fig. 16).

Magdala red.

$I_\lambda d\lambda$ = 200 ergs/cm².sec.

$I_\lambda\, d\lambda\, a_{\lambda_1}\, d\lambda_1$ at concentrations of

Wave-lengths.	M/1200.	M/3000.	M/6000.	M/12,000.	M/24,000.	M/48,000.
560	...	...	...	0·02	0·024	0·028
570	...	...	0·04	0·073	0·11	0·128
580	...	0·05	0·078	0·112	0·161	0·166
590	0·035	0·072	0·087	0·138	0·172	0·171
600	0·072	0·078	0·09	0·122	0·121	0·128
610	0·052	0·066	0·076	0·10	0·10	0·10
620	0·05	0·056	0·076	0·09	0·092	0·092
630	0·056	0·06	0·076	0·08	0·08	0·08
640	0·051	0·056	...	...	...	...
Conc. ...	M/1200	M/3000	M/6000	M/12,000	M/24,000	M/48,000
$I_\lambda\, d\lambda\, a$ ...	29·6	41·6	54·1	68·7	79·6	82·2
a ...	0·148	0·208	0·27	0·343	0·398	0·411

Remark—A subsidiary maximum is observed at 630 μμ at a concentration M/1200. This maximum is shifted towards the violet end along with the principal maximum 600μμ with dilution.

TABLE XIII (*vide* Fig. 17).

Rhodamine B.

$I_\lambda\, d\lambda$ = 220 ergs/cm^2./sec.

$I_\lambda\, d\lambda a_{\lambda_1}\, d\lambda_1$ at concentrations of

Wave-lengths.	M/1000.	M/2500.	M/5000.	M/10,000.
540 μμ	...	...	0·0125	0·03
550	...	...	0·063	0·09
560	0·023	0·09	0·12	0·156
570	0·05	0·115	0·19	0·214
580	0·075	0·13	0·225	0·23
590	0·10	0·11	0·14	0·15
600	0·08	0·09	0·10	0·10
610	0·065	0·07	0·075	...
Conc. ...	M/1000	M/2500	M/5000	M/10,000
$I_\lambda\, d\lambda\, a$ ...	34·7	70·5	100	109
a ...	0·158	0·32	0·455	0·495

TABLE XIV (*vide* Fig. 18).

PhosphineG.

$I_\lambda\, d\lambda$ = 200 ergs/cm.^{2}/sec.

$I_\lambda\, d\lambda\, a_{\lambda_1}\, d\lambda_1$ at concentrations of

Wave-lengths.	M/1300.	M/3250.	M/6500	M/13,000
480 μμ	...	...	0·0075	0·009
490	...	...	0·021	0·0235
500	...	0·016	0·029	0·03
510	...	0·0175	0·031	0·033
520	0·01	0·0185	0·032	0·035
530	0·0115	0·02	0·028	0·031
540	0·013	0·015	0·023	0·026
550	0·01	0·0125	0·018	0·02
560	0·0075	0·01	0·014	0·015
570	0·00575	0·0075	0·012	0·0125
580	0·0044	0·06	...	...
Conc. ...	M/1300	M/3250	M/6500	M/13000
$I_\lambda\, d\lambda\, a$ ...	6·5	13·07	22·0	24·0
a ...	0·0325	0·065	0·11	0·12

At concentrated solutions perhaps there are two maxima between 490 to 540μμ which overlap.

TABLE XV (*vide* Fig. 19).

Phloxine.

$I_\lambda\, d\lambda = 200$ ergs/cm.^{2}/sec.

Wave-lengths.	$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of M/1000.	M/5000.	M/10,000.	M/20,000
530$\mu\mu$	...	0·0224	0·0275	0·032
540	...	0·06	0·074	0·087
550	...	0·10	0·12	0·15
560	0·005	0·075	0·10	0·14
570	0·02	0·063	0·083	0·081
580	0·031	0·060	0·06	0·06
590	0·0367	0·054	0·054	0·054
600	0·3	...	...	...
610	0·0234	...	...	...
Conc. ...	M/1000	M/5000	M/10,000	M/20,000
$I_\lambda\, d\lambda\, a$ ...	11·85	39·3	48·0	54·0
a ...	0·059	0·197	0·24	0·27

TABLE XVI (*vide* Fig. 20).

Acriflavine.

$I_\lambda\, d\lambda = 80$ ergs/cm.^{2}/sec.

Wave-lengths.	$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$ at concentrations of M/500.	M/1000.	M/2000.	M/4000.	M/8000.	M/160,00.
480$\mu\mu$	0·005	0·006	0·01	0·0125	0·016	0·016
490	0·106	0·0144	0·019	0·0275	0·045	0·045
500	0·0186	0·0234	0·031	0·0446	0·051	0·051
510	0·022	0·0327	0·033	0·039	0·039	0·039
520	0·025	0·0257	0·030	0·035	0·035	0·035
530	0·0217	0·0227	0·0234	0·024	0·024	0·024
540	0·0177	0·0170	0·017	0·018	0·019	0·019
550	0·015	0·017	0·018	0·018	0·015	0·015
560	0·0105	0·012	0·0125	0·0125	0·0125	0·0125
Conc. ...	M/500	M/1000	M/2000	M/4000	M/8000	M/16,000
$I_\lambda\, d\lambda\, a$...	15·1	20·0	22·3	27·15	29·7	29·7
a ...	0·19	0·25	0·28	0·35	0·38	0·38

A subsidiary maximum at about 550 $\mu\mu$ was observed along with the main peak at 500 $\mu\mu$.

The following values for the maximum fluorescence yields of dyestuffs in alcoholic solutions were observed, when the exciting wave-length was 365$\mu\mu$.

Dyestuffs.	Maximum fluorescence yields.	Dyestuffs.	Maximum fluorescence yields.
1. Fluorescein	0·72	6. Magdala red	0·415
2. Eosin	0·68	7. Rhodamine B	0·5
3. Rhodamine 6G	0·75	8. Phosphine 2G	0·125
4. Rhodulin orange	0·68	9. Phloxine	0·28
5. Benzoflavine	0·66	10. Acriflavine	0·40

The experimental results confirm the general observation that the fluorescence yields remain constant at very low concentrations but beyond this limiting concentration, the yields fall off very rapidly with increase in concentrations.

FIG. 19.

Phloxine.

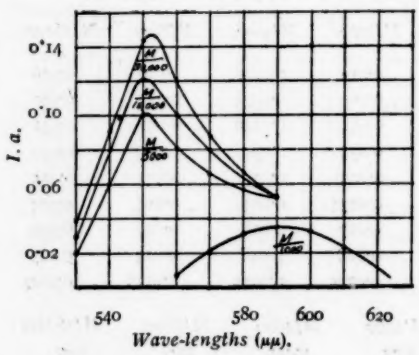
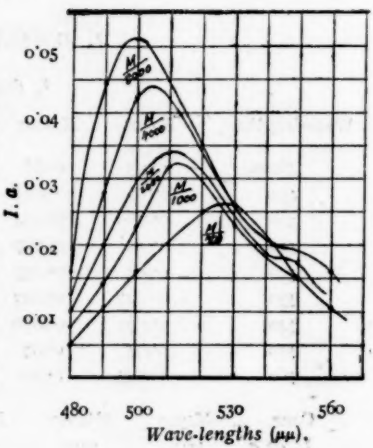


FIG. 20.

Acriflavine.



We have observed that with increase of concentrations, the maxima or the maximum of the fluorescence bands are always shifted towards the red end of the spectrum.

Fluorescence Yields as a Function of Wave-lengths of Exciting Light.

TABLE XVII (*vide* Fig. 21).

Rhodamine 6 G.

Conc. = $M/20{,}000$.

Wave-lengths of exciting light ($\mu\mu$) ...	313	365	436	546
Incident energy (ergs/ cm.^{2}./sec.) ...	6	21	10	25
Wave-lengths.	$I_\lambda\, d\lambda a_{\lambda_1} d\lambda_1$.			
540 $\mu\mu$	0·003	0·016	0·007	0·0172
550	0·0065	0·022	0·014	0·034
560	0·007	0·029	0·0138	0·04
570	0·005	0·0245	0·012	0·032
580	0·004	0·0184	0·0085	0·025
590	0·003	0·0162	0·007	0·0188
600	0·002	0·012	0·005	0·014
610	0·0015	0·008	0·004	0·0108
620	...	0·006	...	0·008
$I_\lambda\, d\lambda\, a$ ...	4·0	14·4	8·1	21·4
a ...	0·66	0·70	0·81	0·86

All the values of $I_\lambda d\lambda a_{\lambda_1} d\lambda_1$ are plotted in the graph.

TABLE XVIII.

Rhodamine B.

Conc. = $M/10{,}000$.

Exciting wave-lengths ($\mu\mu$) ...	313	365	546	577, 579
$I_\lambda\, d\lambda$ (ergs/ cm.^{2}/sec.) ...	6·36	19·6	24·5	15·5
$I_\lambda\, d\lambda\, a$	2·8	9·8	12·9	6·2
a	0·44	0·50	0·53	0·40

TABLE XIX.

Magdala red.

Concentration = $M/20{,}000$

Exciting wave-lengths ($\mu\mu$)	...	313	365	546	577, 579
$I_\lambda\, d\lambda$ (erg./cm.²/sec.)	...	6	13·0	20·0	8·0
$I_\lambda\, d\lambda$ a	...	2·1	5·3	10·3	4·0
a	...	0·35	0·40	0·52	0·50

FIG. 21.

Rhodamine 6 G.

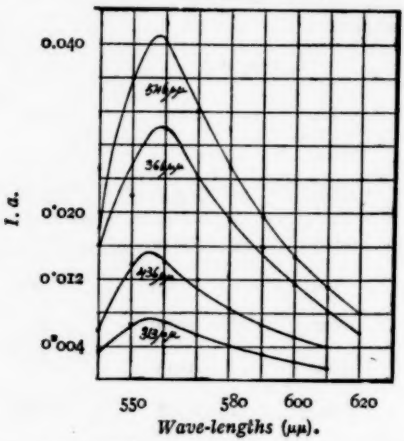


FIG. 22(*a*).

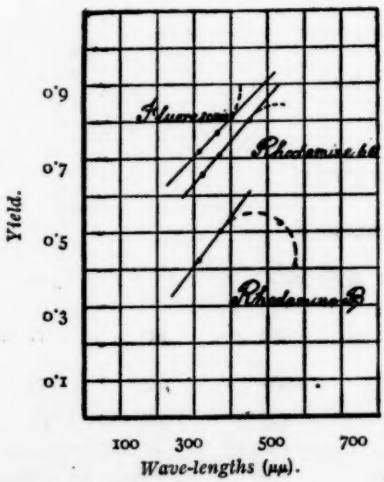
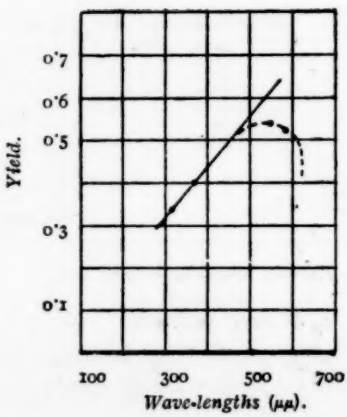


FIG. 22(*b*).

Magdala red.



It would be evident from the above experimental facts that the structure of fluorescence band does not materially alter with change of wave-lengh of exciting light.

Though our results (Fig. 22) are not very exhaustive and only limited to a few wave-lengths, we may safely deduce the conclusion that the fluorescence yields of dyestufis are proportional to wave-lengths of exciting light up to a maximum of visible absorption band. Within the visible absorption band, the yields slightly increase or remain constant in accordance with the observation of previous investigators. The yields rapidly fall off at the approach of maximum of fluorescence band and finally become zero.

Molecular Conductivities of some of the Fluorescent Dyestuffs in Alcoholic Solution.

TABLE XX (*vide* Fig. 23).

Rhodamine B.

Temp. = 29°.

Mol. conc. (*C*).	$\sqrt{C}$.	Mol. conducty.	Mol. conc. (*C*).	$\sqrt{C}$.	Mol. conducty.
0·002	0.0447	5·72	0·00025	0·0158	6·065
0·001	0·0316	6·005	0·000125	0·01118	6·05
0·0005	0·02236	6·11	0·0000625	0·0079	6·00

TABLE XXI (*vide* Fig. 24).

Eosin.

Temp. = 30°.

Mol conc. (*C*).	$\sqrt{C}$.	Mol. conducty.	Mol. conc. (*C*).	$\sqrt{C}$.	Mol. conducty.
0·002	0·0447	40·0	0·000125	0·0118	63·26
0·001	0·0316	46·71	0·0000625	0·0079	67·0
0·0005	0·02236	52·7	0·00003125	0·0056	70·0
0·00025	0·0158	58·25			

TABLE XXII (*vide* Fig. 24).

Acriflavine.

Temp. = 29·5°.

Mol. conc.	$\sqrt{C}$.	Mol. conducty.	Mol. conc.	$\sqrt{C}$.	Mol. conducty.
0·002	0·0447	36·5	0·000125	0·01118	48.3
0·001	0·0316	40·4	0·0000624	0·0079	49·7
0·0005	0·02236	43·8	0·00003125	0·00562	51·05
0·00025	0·0158	46·4			

TABLE XXIII (*vide* Fig. 25).

Magdala red.

Temp. = 30°.

Mol. conc.	$\sqrt{C}$.	Mol. conducty.	Mol. conc.	$\sqrt{C}$.	Mol. conducty.
0·002174	0·04672	23·4	0·0002717	0·01648	27·4
0·001087	0·03297	24·68	0·0001359	0·01166	28·3
0·0005435	0·0233	26·3	0·00006793	0·00824	29·2

TABLE XXIV (*vide* Fig. 23).

Rhodamine 6 G.

Temp-= 30°.

Mol. conc.	$\sqrt{C}$.	Mol. conducty.	Mol. conc.	$\sqrt{C}$.	Mol. conducty.
0·001476	0·03872	28.4	0·0000937	0·009675	33·48
0.00075	0·02737	30·26	0·00004685	0·00684	34·27
0·000375	0·01933	31·56	0·00002342	0·00483	34·87
0·0001875	0·01366	32·58			

It will appear from the graphs that when the molecular conductivities of dyestuffs in alcohol are plotted against square root of molar concentrations, they do not give a linear relation, even at a concentration of 0·01*M* as is expected from Debye and Hückel's theory. It has sometimes been found that the molecular conductivities pass through a maximum. The signficance of this abnormal behaviour has been discussed by various investigators.

FIG. 23.

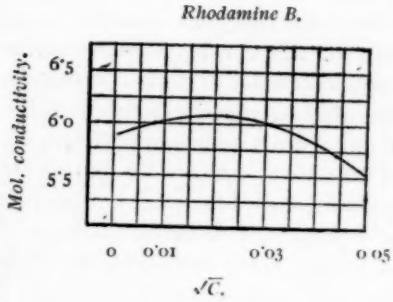


FIG. 24.

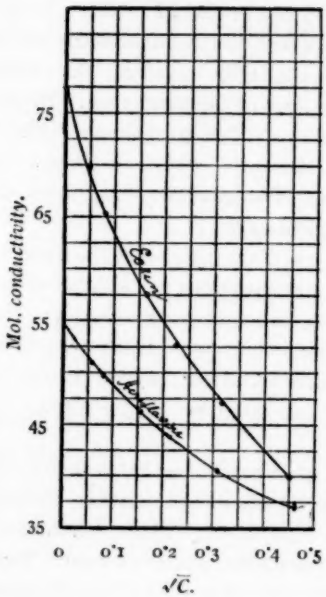
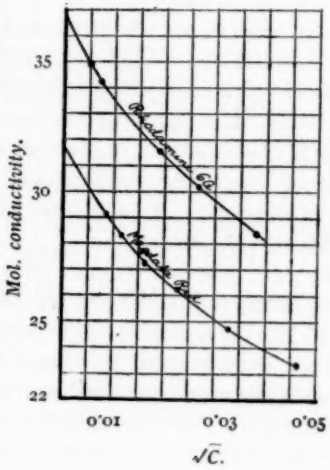


FIG. 25.



The Mechanism of Quenching of Fluorescence.

The quenching of fluorescence of dyestuffs with increase in concentration or by addition of salts has been explained by various investigators as due to deactivating collisions of second kind.

Lewschin (*Z. Physik*, 1927, **43**, 230) found that for rhodulin orange and fluorescein in sucrose, the values of specific fluorescent capacity are practically the same as in ordinary liquids of small viscosity. From this result, Lewschin concluded that the mechanism for quenching for fluorescence must be the same in solid and in liquid solution and therefore practically independent of viscosity. Banow (*Z. Physik*, 1929, **58**, 811)

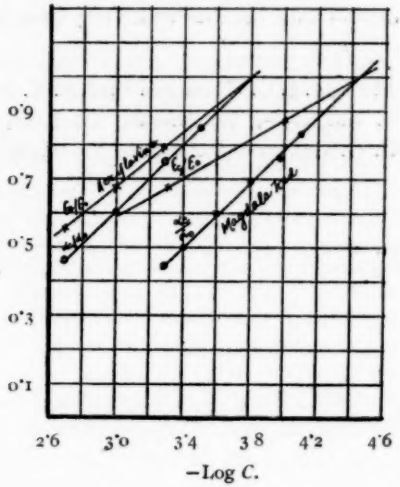
confirmed this observation and has further shown that a three hundred fold increase in viscosity scarcely affects the extinction curves. Further evidences against the deactivation collision, have been supplied by Bouchard (*loc. cit.*) who showed that the enormous effect of glycerine on the quenching coefficients is due to specific solvent effects and if due corrections are made, the effects of viscosity on the quenching coefficient is negligible (8-16%). Hence it is not unreasonable to suppose that the mechanism for quenching of fluorescence is almost the same in liquid and in viscous media. Lewschin (*loc. cit.*) studied the effect of temperature on specific fluorescent capacity in concentrated solutions. Though temperature increases the number of collisions, the fluorescence yields increases at the same time. Bouchard (*loc. cit.*) has confirmed the above observations which go against a collision of second kind as means of quenching. Banow (*Z. physikal. Chem.*, 1933, **153A**, 172) has further shown that increase of temperature, has, sometimes a decreasing effect on the quenching of fluorescence.

F. Perrin (*Compt. Rend*, 1931, **192**, 1727) suggested that the more rapid decrease of fluorescence with increasing concentrations and smaller optimum concentration of solutions of fluorescein, when aqueous potassium chloride solution is used as solvent in place of pure water, supports the view that the decrease of fluorescence on increasing concentrations beyond the optimum, is due to association of molecules, as conceived by Walter (*Wied. Ann.*, 1888, **34**, 22; 1889 **36**, 518).

We have observed in our work a close parallelism between the change of molar absorption coefficients with concentrations and change of fluorescence yields. It is interesting to note that in very dilute solution, where Beer's law seems to hold, the fluorescence yields remain constant. Further increase in concentration diminishes the yields as well as absorption capacity per molecules. This leads us to think that the same causes, which diminish the yields, would also account for the deviation of Beer's law in the direction indicated by our experiments. It will be difficult to explain on purely collision hypothesis the change of absorption coefficients to the extent actually observed by us. We are, thus, inclined to look for an explanation of the observed facts in the formation of micelle ions.

In Figure 26 the values for absorption capacity ϵ_c/ϵ_o (where ϵ_c is the molar absorption coefficient at concentration C, ϵ_o its value at infinite dilution) and fluorescent capacity a_c/a_o (where a_c is the fluorescent yield at concentration C and a_o is the fluorescent yield at infinite dilution) are plotted against log C. It is interesting to note that all these straight lines meet at the same point corresponding approximately to critical concentrations. It is not unreasonable to assume that causes which are

FIG. 26.



responsible for variation of absorption capacity with concentration, may also account for the variation of fluorescence yields with concentrations.

We have observed that if molecular conductivities of fluorescent dyes be plotted against square root of molar concentrations, they give curves similar in nature to those obtained by Moillet, Collie and Robinson (*Trans. Faraday Soc.*, 1935, **31**, 120) in the case of methylene blue, *meta*benzopurpurine and long chain paraffin salts. In view of the similarity of curves obtained, we are tempted to believe in agreement with these investigatiors that at concentrated solutions of dyestuffs, ionic micelles are formed and these micelles are gradually broken into simpler forms and finally into ions, as we increase the dilution.

It may be pointed out that the molecular conductivities plotted against the square root of concentrations give curves which may be divided into two parts. The tangential straight lines drawn to these curves meet at a point corresponding to a certain concentration. It is interesting to note that these concentrations or the concentrations corresponding to maxima of conductivity coincide with the concentrations after which rapid quenching of fluorescence begins. It also suggests that fluorescence is largely dependent on the state of ions.

It is premature to draw any picture of micelle formations unless we have some data on transport number of dye ions. Further investigations on the transport number of fluorescent dyes are in progress with a view to throw light on the constitution of ionic micelles which may exist in solution.

My thanks are due to Prof. J. C. Ghosh and Prof. S. N. Bose for their kind interest, to Prof. J. N. Mukherjee, for lending his König-Marten spectrophotometer and to Mr. H. Mukheriee for lending his conductivity apparatus.

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VISCOSITY AND DENSITY OF AQUEOUS SOLUTIONS OF MERCURIC CHLORIDE AT 35°.

By Shivanandan Prasad, Amritansu Sekhar Chakravarti and Balbhadra Prasad.

Viscosity and density of aqueous solutions of mercuric chloride have been measured at 35° from 0·001534 to 0·2652 g. mole/litre (almost saturated solution) with a viscometer in which neither kinetic correction nor surface tension correction was necessary. The density increases linearly with concentration throughout the entire range and can be represented by the equation $\rho = 0{\cdot}9941 + 0{\cdot}228\,c$. Viscosity too increases linearly with concentration for dilute solutions ($\eta/\eta_0 = 1 + 0{\cdot}125c$), but at a concentration near about $M/10$ and above the viscosity begins to increase more rapidly. This linear variation of viscosity with concentration in dilute solutions is taken as a proof of the behaviour of mercuric chloride as almost a non-electrolyte in aqueous solutions.

The viscosity of dilute solutions of non-electrolytes was measured by one of the authors (*J. Indian Chem. Soc.*, 1935, **12**, 499) who showed that in dilute solutions, viscosity increases linearly with concentration. Mercuric chloride behaves as a very weak electrolyte in both conductivity and electromotive force measurements and so, it was expected to behave more or less as a non-electrolyte in viscosity measurements. The experiments were undertaken to test the validity of this expectation.

Experimental.

The thermostat used was of the same type as that described in the paper referred to above. The temperature was kept at 35° ± ·005. Viscosity was measured with the same kind of viscometer, except that the mark below the bulb on the capillary side was made just above the capillary tube at a place where the bore was several times greater than the bore of the capillary. The viscometer was fixed in a frame, so that a number of adjustments described in the previous paper were rendered unnecessary. For observing the level of the liquid in the wider limb, a telescope giving a linear magnification of three at a distance of two feet was used. Five readings for time were taken in case of every solution and the mean was used in calculating viscosity. The bore of the capillary tube in the viscometer used was about 0·28 mm. and its length about 10 cm. The volume of the bulb above the capillary tube was about 5 c.c. and the time of transpiration about 30 minutes.

Density measurements were made with a pyknometer of about 20 c. c. capacity, provided with stoppers and ending in vertical capillary limbs of about 1 mm. bore (instead of the usual type with horizontal limbs) so that the whole of the liquid could be kept well under the surface of water in the thermostat. After the liquid in the pyknometer had attained the temperature of the bath, the level was adjusted with a fine platinum syringe to marks made near about the middle of each capillary limb. Two determinations were made with the pyknometer in each case and the results never differed by more than 1 mg. Double distilled water was used in making all the solutions.

No surface tension correction was necessary on account of the design of the viscometer. No kinetic correction was necessary, as found by the method suggested by Barr (A Monograph of Viscometry, Oxford, 1931, p. 127).

In the following table, the concentrations of solutions of mercuric chloride, their density and viscosity are given. By concentration is meant g. moles/litre and by viscosity the relative viscosity, *i.e.*, viscosity of solution/viscosity of water at the same temperature (35°).

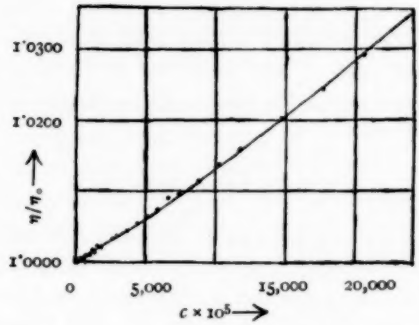
TABLE I.

Conc. × 10^5.	Density.	Viscosity.	Conc. × 10^5.	Density.	Viscosity.
0	0·9941	1·0000	2950·0	1·0008	1·0037
153·4	0·9944	0·9998	3699·0	1·0023	1·0045
302·0	0·9949	1·0004	4420·0	1·0039	1·0054
452·0	0·9951	1·0003	5152·0	1·0054	1.0064
593·8	0·9955	1·0004	5900·0	1·0072	1·0074
749·2	0·9958	1·0010	6630·0	1·0089	1·0090
882·5	0·9961	1·0009	7367·0	1·0105	1·0095
1033·0	0·9963	1·0009	8840·0	1·0137	1·0113
1182·0	0·9969	1.0017	10314·0	1·0169	1·0138
1329·0	0·9970	1·0013	11787·0	1·0201	1·0157
1526·0	0·9976	1·0023	14733·0	1·0268	1·0203
1768·0	0·9980	1·0021	17679·0	1·0333	1·0245
2063·0	0·9987	1·0029	20626·0	1·0407	1·0292
2357·0	0·9994	1·0034	23557·0	1·0472	1·0342
2652·0	1·0000	1·0033	26520·0	1·0538	1·0378

The maximum possible error in the values of viscosity (η/η_0), as found by assuming a maximum error of 0·2 second in the time of transpiration and 0·001 g. in weights comes out to be 0·0003, but the actual error in most cases should be much less.

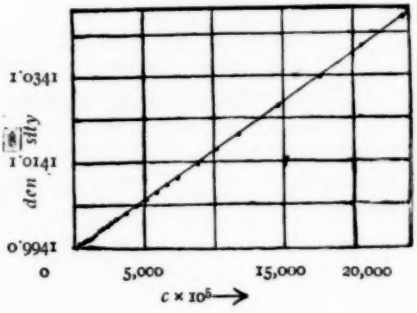
In Fig. 1, the viscosity of the solutions is plotted against concentration.

FIG. 1.



In the following figure, density of all the solutions is plotted against concentration.

FIG. 2.



DISCUSSION.

An examination of the results given in the table as well as those plotted in Fig. 1 shows that the variation of viscosity is so small that at low concentrations, the difference between the viscosity of two successive solutions is within the limits of the possible experimental error (0·0003).

To test whether mercuric chloride behaves as an electrolyte or non-electrolyte, η/η_0 was plotted against c. A straight line passing through the origin was obtained by the method of least squares up to a concentration of 0·08840 g. mole per litre. The viscosity of seventeen out of twenty-three solutions, thus plotted, could be represented by the equation, $\eta/\eta_0 = 1 + 0{\cdot}125c$, within the possible experimental error. In four cases, the difference between the observed and the calculated results was 0·0004, in one case 0·0005 and in another 0·0007. This very fact would be enough to show that mercuric chloride behaves as a non-electrolyte, but in order to make sure, the A and B values of Jones and Dole equation (*J. Amer. Chem. Soc.*, 1929, **51**, 2950; Falkenhagen and Vernon, *Phil. Mag.*, 1932, **14**, 537),

$$\eta/\eta_0 = 1 + A\sqrt{c} + Bc$$

were found by plotting $\eta/\eta_0 - 1/\sqrt{c}$ against $\sqrt{c}$. The intercept on the ordinate gives A and the slope of the line gives B. It is found that a very small negative value can be given to A. No positive value is possible. The results are equally well represented if $A = 0$, which means that mercuric chloride is a non-electrolyte. Moreover, the negative value does not seem to have any significance.

Density (ρ) of the solutions varies linearly with concentration from the lowest to highest concentrations and can be represented to an accuracy of 0·1% by the equation $\rho = 0{\cdot}9941 + 0{\cdot}228c$.

In conclusion two of us (S. N. P. and A. S. C.) wish to express their gratitude to the Government of Orissa in the Ministry of Education for the grant of Research Scholarships which enabled them to take part in the investigation.

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COMPOUNDS OF HEXAMETHYLENETETRAMINE WITH SIMPLE AND DOUBLE SALTS OF COBALTICYANIC ACID AND THE NATURE OF RESIDUAL AFFINITY.

By Basanta Kumar Ghosh.

Preparation, properties and the composition of the compounds of hexamine with the simple and double cobalticyanides have been described.

The capacity of hexamethylenetetramine to form additive compounds with numerous simple and complex salts is well-known. Its compounds with many complex metallocyanic acids and their salts have been investigated by Rây and his co-workers in order to explore the nature of residual affinity and to examine the possibility of their utilisation for analytical purposes (Rây and Sarkar, *J. Chem. Soc.*, 1921, 3903; Rây and Das-Gupta, *J. Indian Chem. Soc.*, 1928, **5**, 519 ; Rây and Sarkar, *Mikrochem. Emich Festschrit*, 1930, 243; Rây and Bakshi, *J. Indian Chem. Soc.*, 1934, **11**, 125). From a study of the composition of the compounds of urotropine with complex ferro- and ferricyanides described by Barbieri (*Gazzetta*, 1930, **60**, 229) as well as with complex thiosulphato-pentacyano-cobaltic salts described by Rây and Bakshi (*loc. cit.*), the latter authors have shown that the capacity for association with water molecules for any particular cyano-complex increases with its simultaneous association with hexamine molecules ; in other words, the partial saturation of residual affinity of the cyano-complexes seems rather paradoxically to intensify the latter. This apparent anomaly was accounted for by Rây and Bakshi as due to an electrostatic character of the residual affinity exerted in these cases. The nature of residual affinity may be regarded here to resemble that of "Field Valency", which is very much akin to what prevails within a crystal between molecules of one and the same substance.

Recently a number of compounds of hexamine with complex nitro-pentacyanides has been prepared by Voyatzakis (*Compt. rend.*, 1936, **203**, 1365). Similar compounds were also prepared by the present author, but the work was left unpublished. Their composition are in close agreement with those of Voyatzakis. The preparation, properties and the composition of the compounds of hexamine with the simple and double cobalticyanides have been described in this paper. A comparison of the composition of these compounds of hexamine and complex cyanides with those of the complex cyanides themselves reveals certain interesting facts. The

capacity of the cyano-complexes for union with water molecules increases on association with hexamine, as already indicated by Rây and Bakshi, in the case of simple cobalticyanides and nitroprussides, but decreases, on the other hand, in the case of double cobalticyanides. This is apparent from the following table. Evidently an alteration in the strength of the electric field around the complex ion is the cause of all these anomalies.

TABLE I.

$X=[Co(CN)_6]'''$. $B=C_6H_{12}N_4$.

Original salt.	Compound with hexamine.
Ba_3X_2, 20 or 22 H_2O	Ba_3X_2. 2·5 B, 23·5 H_2O
Sr_3X_2, 20 H_2O	Sr_3X_2, 2·5 B, 28 H_2O
K_3X	K_3X, B, 6·5 H_2O —Rây and Sarkar (*loc. cit*).
BaKX, 11 H_2O	BaKX, 1·5B, 6 H_2O
CaKX 9H_2O	CaKX, 1·5B, 5H_2O
SrKX, 9 H_2O	SrKX, 1·5B, 8 H_2O
$Ba(NH_4)X$, 11 H_2O	$Ba(NH_4)X$, 2B 5·5 H_2O
$Ca(NH_4)X$. 10 H_2O	$Ca(NH_4)X$, 1·5B, 7·5 H_2O
$Sr(NH_4)X$, 10 H_2O	$Sr(NH_4)X$, 2B, 3 H_2O

For the compounds with nitroprussides, where $X=[Fe(CN)_5NO]''$.

CaX. 4H_2O	CaX, 2B, 8 H_2O	—Voyatzakis (*loc. cit.*)
BaX, H_2O or 3 H_2O	BaX 2B, 4 H_2O	
Na_2X, 2 H_2O	Na_2X, 2B, 4 H_2O	
K_2X	K_2X, 2B, 3 H_2O	

EXPERIMENTAL.

Barium Salt and Urotropine.—A moderately concentrated solution of hexamethylenetetramine was added, drop by drop, with constant stirring to a concentrated solution of barium cobalticyanide, prepared by double decomposition from silver cobalticyanide and barium chloride. The white crystalline precipitate formed was filtered, washed at first with 5% urotropine

solution, then with 50% alcohol and finally with absolute alcohol. The crystals were afterwards dried in air. {Found : Ba, 25·6 ; Co, 7·29 ; $C_6H_{12}N_4$, 21·75. $Ba_3[Co(CN)_6]_2$, 2·5 $C_6H_{12}N_4$, 23·5 H_2O requires Ba, 25·52 ; Co, 7·3 ; $C_6H_{12}N$, 21·67 per cent}.

Calcium Salt and Urotropine.—Prepared like the barium salt from urotropine and calcium cobalticyanide ; the latter was obtained by double decomposition between silver cobalticyanide and calcium chloride. The white crystalline precipitate was filtered, washed and dried as before. {Found : Ca, 10·17 ; Co, 10·01 ; $C_6H_{12}N_4$, 17·82. $Ca_3[Co(CN)_6]_2$, 1·5, $C_6H_{12}N_4$, 23·5 H_2O requires Ca, 10·14 ; Co, 9·97 ; $C_6H_{12}N_4$, 17·75 per cent}.

Strontium Salt and Urotropine.—Prepared from strontium cabalticyanide and urotropine as in the previous case. The strontium cobalticyanide was prepared from silver cobalticyanide and strontium chloride. {Found : Co, 7·66 ; Sr, 17·08 ; $C_6H_{12}N_4$, 22·52. $Sr_3[Co(CN)_6]_2$, 2·5 $C_6H_{12}N_4$, $28H_2O$ requires Co, 7·62 ; Sr, 17·0 ; $C_6H_{12}N_4$, 22·62 per cent}.

Barium Potassium Salt and Urotropine.—A saturated solution of potassium cobalticyanide (3·3 g.) was mixed with requisite amount of barium chloride (2·2 g.) and a little potassium chloride (0·4 g.) solution. On adding a concentrated solution of hexamine to the mixture, a white crystalline precipitate separated. These were drained, washed first with 5% urotropine solution, then with 50% alcohol and finally with absolute alcohol. The crystals were dried in air. The substance is moderately soluble in water. {Found : Ba, 19·0 ; Co, 8·12 ; K, 5·45 ; $C_6H_{12}N_4$, 29·7 $BaK[Co(CN)_6]$, 1·5 $C_6H_{12}N_4$, $6H_2O$ requires Ba, 19·37 ; Co, 8·3 ; K, 5·5 ; $C_6H_{12}N_4$, 29·6 per cent}.

Magnesium Salt and Urotropine.—Saturated solutions of magnesium cobalticyanide and urotropine, on being mixed together, gave a white gelatinous precipitate. The magnesium cobalticyanide was prepared from silver cobalticyanide and magnesium chloride. The precipitate was washed as before and dried on the porous plate. It was finally kept over concentrated H_2SO_4. {Found : Co, 9·1 ; Mg, 5·5 ; $C_6H_{12}N_4$, 32·6 ; H_2O, 28·54. $Mg_3[Co(CN)_6]_2$, $3C_6H_{12}N_4$, $21H_2O$ requires Co, 9·0 ; Mg, 5·5 ; $C_6H_{12}N_4$, 32·3 ; H_2O, 29 per cent}.

Calcium Potassium Salt and Urotropine.—Potassium cobalticyanide (3·3 g.), calcium chloride (1 g.) and potassium chloride (0·4 g.) were dissolved in the least quantity of water. A strong solution of hexamine was added to this solution with constant stirring. The white crystalline precipitate was filtered, washed and dried as in the previous case. {Found :

Ca, 6·7 ; Co, 9·94 ; K, 6·59 ; $C_6H_{12}N_4$, 35·5. $CaK[Co(CN)_6]$, 1·5 $C_6H_{12}N_4$, $5H_2O$ requires Ca, 6·73 ; Co, 9·93 ; K, 6·57 ; $C_6H_{12}N_4$, 35·35 per cent}.

Strontium Potassium Salt and Urotropine.—Potassium cobalticyanide (3·3 g.), strontium chloride (2·5 g.) and a little potassium chloride were dissolved in the least amount of water. The solution was then treated with a concentrated solution of hexamine in slight excess. The precipitate of white crystals was filtered, washed and dried as before in air. {Found : Co, 8·6 ; K, 5·69 ; Sr, 12·74 ; $C_6H_{12}N_4$, 30·04. $SrK[Co(CN)_6]$, 1·5 $C_6H_{12}N_4$, $8H_2O$ requires Co, 8·48 K, 5·6, Sr, 12·6; $C_6H_{12}N_4$, 30·18 per cent}.

Magnesium Potassium Salt and Urotropine.—Potassium cobalticyanide (3·3 g.), magnesium chloride (2 g.) and a little potassium chloride were dissolved in water. A white gelatinous precipitate was obtained by adding a concentrated solution of hexamine in slight excess to this solution. This was filtered, washed and dried as in the case of the previous magnesium compound. {Found : Co, 9·3 ; K, 6·14 ; Mg, 3·8 ; $C_6H_{12}N_4$, 22·1 ; H_2O, 34. $MgK[Co(CN)_6]$, $C_6H_{12}N_4$, $12H_2O$ requires Co, 9·3 ; K, 6·15 ; Mg, 3·78 ; $C_6H_{12}N_4$, 22·0 ; H_2O, 34·0 per cent}.

Barium Ammonium Salt and Urotropine.—Ammonium cobalticyanide, prepared from silver cobalticyanide, barium chloride and a little ammonium chloride were dissolved in the least amount of water and the solution was treated with a strong solution of hexamine. The white crystalline precipitate was washed, and dried as before. {Found : Ba, 18·4; Co, 7·9; NH_4, 2·4. $C_6H_{12}N_4$, 37·6. $Ba(NH_4)[Co(CN)_6]$, $2C_6H_{12}N_4$, 5·5 H_2O requires Ba, 18·3; Co, 7·87; NH_4, 2·4; $C_6H_{12}N_4$, 37·3 per cent}.

Calcium Ammoniam Salt and Urotropine.—The compound was prepared from the ammonium cobalticyanide, ammonium chloride, calcium chloride, and urotropine in the same way as the previous compound. {Found : Ca, 6·5; Co, 9·58; NH_4, 2·9; $C_6H_{12}N_4$, 34·28. $Ca(NH_4)[Co(CN)_6]$, $1·5C_6H_{12}N_4$, 7·5 H_2O requires Ca, 6·48; Co, 9·55; NH_4, 2·9; $C_6H_{12}N_4$, 34·0 per cent}.

Strontium Ammonium Salt and Urotropine.—The substance was prepared from the ammonium cobalticyanide, ammonium chloride, strontium chloride and hexamine like the previous compound. {Found : Co, 8·98; NH_4, 2·75; Sr, 13·36; $C_6H_{12}N_4$, 42·5. $Sr(NH_4)[Co(CN)_6]$, 2 $C_6H_{12}N_4$, $3H_2O$ requires Co, 9·0 ; NH_4, 2·75 ; Sr, 13·38 ; $C_6H_{12}N_4$, 42·7 per cent}.

Magnesium Ammonium Salt and Urotropine.—Magnesium chloride, a little ammonium chloride and hexamine were added to a concentrated solution of ammonium cobalticyanide, when a white mass was obtained. It was

washed and dried as in the case of the other magnesium compounds. {Found : N, 25·38; Co, 9·8; Mg, 4·1; H_2O, 34·0. $Mg(NH_4)[Co(CN)_6]$, $C_6H_{12}N_4$, 11·5 H_2O requires N, 25·5 ; Co, 9·77 ; Mg, 4·0 ; H_2O, 34·2 per cent}.

Barium Sodium Salt and Urotropine.—Sodium cobalticyanide (3·2 g.), barium chloride (2·3 g.) and sodium chloride (0·4 g.), were dissolved in the least quantity of water. The white crystals, precipitated by the addition of concentrated solution of hexamine, were washed and dried in air as before. {Found : Ba, 18·3 ; Co, 7·85 ; Na, 3·1 ; $C_6H_{12}N_4$, 37·85. $BaNa[Co(CN)_6]$, $2C_6H_{12}N_4$, $5H_2O$ requires Ba, 18·4 ; Co, 7·9 ; Na, 3·07 ; $C_6H_{12}N_4$, 37·4 per cent}.

Calcium Sodium Salt and Urotropine.—This compound was obtained as a white crystalline precipitate when a concentrated solution of hexamethylenetetramine was added to a solution of sodium cobalticyanide (3·2 g.), calcium chloride (1 g.) and a little sodium chloride. This was washed and dried as before. {Found : Ca, 5·25; Co, 7·55; Na 2·8; $C_6H_{12}N_4$, 45·15. $CaNa[Co(CN)_6]$, $2·5C_6H_{12}N_4$, $8H_2O$ requires Ca, 5·18 ; Co, 7·64 ; Na, 2·98 ; $C_6H_{12}N_4$, 45·3 per cent}.

Strontium Sodium Salt and Urotropine.—A white crystalline precipitate was obtained by adding a strong solution of hexamine to a solution of sodium cobalticyanide (3·2 g.), strontium chloride (2·6 g.) and sodium chloride (0·4 g.). The crystals were filtered, washed and dried as in the previous cases. {Found : Co, 7·82 ; Na, 3·0 ; Sr, 11·8 ; $C_6H_{12}N_4$, 37·9. $SrNa[Co(CN)_6]$, $2C_6H_{12}N_4$, 7·5 H_2O requires Co, 7·96 ; Na, 3·1 ; Sr, 11·83 ; $C_6H_{12}N_4$, 37·8 per cent}.

Magnesium Sodium Salt and Urotropine.—Sodium cobalticyanide (3·2 g.), magnesium sulphate (2·4 g.) and a little sodium chloride were dissolved in the least quantity of water. On adding a strong solution of urotropine to the mixture, a white gelatinous precipitate was obtained. It was washed and dried as already described in the cases of other magnesium compounds. {Found : Co, 8·42 ; Mg, 3·35 ; Na, 3·29 ; $C_6H_{12}N_4$, 29·6 ; H_2O, 32·9. $MgNa[Co(CN)_6]$, $1·5\ C_6H_{12}N_4$, $13H_2O$ requires Co, 8·35 ; Mg, 3·4; Na, 3·25; $C_6H_{12}N_4$, 29·74; H_2O, 33·1 per cent}.

Method of Analysis.—In the case of barium compounds, barium was estimated as sulphate by precipitation with dilute sulphuric acid from solutions of the compounds. The filtrate was decomposed with concentrated H_2SO_4 and the cobalt precipitated as sulphide. The latter was estimated as sulphate after conversion. In the filtrate from the cobalt sulphide, sodium and potassium were estimated as sulphate.

Total nitrogen was estimated by decomposing the compounds with concentrated H_2SO_4 and then distilling the ammonia with caustic soda (Kjeldahl's method). Nitrogen present as cyanide was calculated from the cobalt content. The percentage of urotropine was then calculated by difference.

With calcium and strontium compounds, the alkaline-earth metals were precipitated as oxalates from solutions of their compounds. These were then converted into sulphate and weighed as such. The filtrate was decomposed with concentrated H_2SO_4. Cobalt and the alkali metals were then estimated as already described above.

With magnesium compounds, magnesium was precipitated and estimated as 8-hydroxyquinoline from solutions of the substances. Cobalt and the alkali metals were estimated as before in the filtrate after decomposing the organic matter with concentrated H_2SO_4.

In the case of alkaline-earth ammonium compounds, ammonia was estimated by distilling the solution of the substances with caustic soda. Percentage of urotropine was then calculated by subtracting the amount of ammoniacal and cyanidic nitrogen from the total nitrogen.

My grateful thanks are due to Prof. P. Rây of the University College of Science, Calcutta, for his kindly suggesting this piece of work to me and for the encouragement I have received from him. I also offer my best thanks to the Principal of Edward College, Pabna, for allowing me all the facilities to carry out this work.

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NATURAL FLAVONES. PART II. ON THE COLOURING MATTERS OF THE BARK OF *OROXYLUM INDICUM*, VENT.

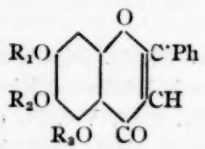
By Prafulla Kumar Bose and Sachindra Nath Bhattacharya.

Oroxylin, the yellow colouring matter of the bark of *Oroxylum indicum*, Vent. was isolated and investigated by Naylor and his collaborators, who ascribed the formula $C_{19}H_{14}O_6$ to the compound. The present authors have been able to prove that Naylor's oroxylin is a mixture of at least three hydroxyflavones, namely, baicalein, 6-methylbaicalein and chrysin. Chrysin and baicalein have been isolated and identified, but 6-methylbaicalein could not be completely freed from chrysin.

Oroxylum indicum, Vent. (N. O. *Bignoniaceae*; Sanskrit: Syonáka, Bengali: Sônâ) has some repute in Hindu medicine (*vide* Kirtikar and Basu, "Indian Medicinal Plants", Ed. 1918, Vol. II, 940). Holmes (*Pharm. J.*, 1890-91, **21**, 257) reported that the bark 'had been found by Dr. Evers to possess definite therapeutic action' in acute rheumatism. It has been reported in *Agricultural Ledger* (No. 6 of 1898) that the seeds can be used against a kind of ring-worm in cattle caused by *Tricophyton tonsurans*. Naylor and Chaplin (*Pharm. J.*, 1890-91, **21**, 257) isolated a yellow crystalline substance, m.p. 228·5-229° from the bark which they called oroxylin. Werner (*Diss.* Erlangen, 1896) appears to have found the same substance in the seeds. Naylor and Dyer (*J. Chem. Soc.*, 1901, **79**, 954) obtained an yield of 0·2% of the product from the bark (m.p. *ca.* 225°) and assigned to it the formula, $C_{19}H_{14}O_6$. Some products of degradation, *e.g.*, benzoic acid, benzaldehyde, phloroglucinol and phthalic acid were also described without proper identification except in the case of the first named substance.

By the process described in the experimental sequel we have isolated the crude colouring matter in an yield of 2·8% and from it about 1·5% of the purified product. Repeated crystallisation of the crude product gave samples having the melting point range of 220°-232° but responding to the tests ascribed to oroxylin by Naylor and Chaplin. From the crude extract, after many trials, we were able to isolate one of the components as the lead salt from an alcoholic solution of the crude extract acidified with acetic acid (*vide* Experimental). The substance, m. p. 262-65°, (brown prisms) was obtained in an yield of 0·5% of the bark used. Besides giving the colour reactions of oroxylin, it gave a voluminous greenish brown precipitate with

chloropentammine cobaltichloride indicating the presence of two -OH groups in *ortho* or *para* positions to one another (*cf.* Shibata and Hattori, *Acta Phytochim.*, 1930, **5**, 117). Sodium amalgam produced an immediate green precipitate in the alcoholic solution of the substance. Hence it has three contiguous hydroxyl groups in the molecule (*cf.* Bargellini, *Gazzetta*, 1919, **49**, 47). The substance was free of methoxyl and its composition agreed with $C_{15}H_{10}O_5$. Alkaline hydrolysis gave benzoic acid and acetophenone; and with diazomethane a trimethyl ether, m. p. 161°, (insoluble in dilute alkali) was formed. From these, it was concluded that the substance probably was identical with baicalein (I, $R_1=R_2=R_3=H$) (Shibata, Iwata and Nakamura, *Acta Phytochim.*, 1923, **1**, 105). Prof. Shizuo Hattori very kindly sent us specimens of baicalein and its trimethyl ether with which we have established the identity of our products by direct comparison and determination of mixed m. p.



(I)

The mother-liquor, after the removal of baicalein lead salt, was treated with hydrogen sulphide, lead sulphide removed and on concentration the solution furnished bright yellow needles. This fraction will be referred to as 'oroxylol' as it did not give any of Naylor and Chaplin's tests of oroxylin. This portion after repeated crystallisation from different solvents gave a fraction which appeared uniform under the microscope but showed a range from 214° to 235° in m. p. Unlike baicalein, the mixture gave no precipitate with chloropentammine cobaltichloride and ammoniacal silver salts. Treatment of an alcoholic solution of the mixture with concentrated hydrochloric acid and magnesium produced an orange colour. Numerous attempts to separate the components of oroxylol, either by fractional precipitation of an alkaline solution or fractional crystallisation from various solvents were not successful. The mixtures obtained at various stages were, therefore, analysed and the carbon content was found to vary from 68·16 to 70·13, hydrogen from 3·96 to 4·96 and methoxyl from 6·54 to 9·76%. Therefore it seemed that the mixture might contain a fairly large percentage of a monomethyl ether of baicalein, which requires C, 67·6, H, 4·23 and OMe, 10·92%. A sample of oroxylol showing m. p. 227-230° was demethylated and baicalein was isolated *via* its lead salt. After the separation of baicalein as its lead salt, the mother-liquor after removal of lead and concentration gave a

crop of pale yellow crystals (m. p. 274-75°) analysing for $C_{15}H_{10}O_4$. This substance was identified as chrysin by comparison with an authentic specimen kindly provided by Dr. K. Venkataraman. Hence it seems that crude oroxylol is a 6 5 : 3·5 mixture of a baicalein monomethyl ether and chrysin. In oroxylol, no chrysin dimethyl ether or baicalein trimethyl ether can be present owing to its complete solubility in dilute aqueous alkali. The mixture may therefore consist of—

(*i*) A monomethyl baicalein (R_1 or R_2 or R_3 being Me in Formula I) + chrysin.
(*ii*) A monomethyl baicalein + a monomethyl chrysin.
(*iii*) A dimethyl baicalein + chrysin.
(*iv*) A dimethyl baicalein + a monomethyl chrysin.

The analytical value of the mixture (m. p. 227-30°) indicates the probability of its being (*i*).

Of the three possible monomethyl baicaleins, the probability is of its being the 6-methyl derivative (I, $R_1 = R_3 = H$; $R_2 = Me$) as the reactions with chloropentammine cobaltichloride and *o*-dinitrobenzene (Bose, *J. Indian Chem. Soc. Sir P. C. Ray Comm. Vol.*, p. 65) point to the absence of *ortho*- or *para*-hydroxyl groups. We are engaged in synthesising 6-methyl ether of baicalein for establishing this point.

We inferred from various considerations that baicalein 6-methyl ether should have a m.p. lower than 230°. Hence some fractions with lower melting points from crude oroxylol were extracted repeatedly with chloroform and analysed. One of these, the fraction m.p. 199-200°, gave OMe less than 9 per cent, whilst a specimen, m.p. 220-223°, gave nearly the theoretical value for 6-methyl ether of baicalein. However, the amount was too small for further purification.

Recently Shah, Mehta and Wheeler (*J. Chem. Soc.*, 1936, 591 ; *Current Sci.*, 1935, **4**, 406) have concluded that oroxylin-A (m. p. 231-32°) isolated from the root-bark of *Oroxylum indicum* is the 6-methyl ether of baicalein.

EXPERIMENTAL.

Isolation of Crude Flavones.—The bark was powdered and extracted with alcohol, and the alcohol removed from the extract under diminished pressure. This operation was carried out by Messrs. Bengal Chemical and Pharmaceutical Works, Ltd., to the authorities of which firm our best thanks are due. The residue was heated with about eight times its weight of water, cooled and filtered (Filtrate A). The residue, after being mixed with sand, was dried and then thoroughly ex-

tracted with hot benzene for 72 hours. The benzene extract was concentrated on the water-bath to a small volume, cooled and filtered. The greenish yellow crystals were washed with petroleum ether to remove adhering oil. The aqueous Filtrate A was repeatedly extracted with ether, the solvent removed and the crystalline residue washed with cold benzene and mixed with the main crude product. The total yield was 2·8% calculated on the amount of bark taken. The m.p. was indefinite (218-260°).

Isolation of Baicalein.—The crude product was dissolved in warm alcohol and filtered to remove a small quantity of insoluble matter. The clear solution was acidified with a little acetic acid, and then a concentrated aqueous solution of lead acetate was gradually added till the precipitation was complete. The dirty brick-red precipitate was filtered off and thoroughly washed with warm alcohol. It was then suspended in hot alcohol and decomposed by passing a current of sulphuretted hydrogen. After the removal of lead sulphide the filtrate, on concentration, deposited brown crystals of crude baicalein. These were purified again *via* the lead salt. Two more crystallisations from alcohol gave yellow-brown prisms, m.p. 262-65°; yield 0·5%. (Found : C, 66·79 ; H, 3·88. $C_{15}H_{10}O_5$ requires C, 66·66 ; H, 3·70 per cent). Mixed with an authentic specimen of baicalein there was no depression of m.p.

Hydrolysis of Baicalein : Isolation of Benzoic Acid and Acetophenone.—0·5 G. of baicalein was heated on the water-bath with 20 c.c. of 10% aqueous potassium hydroxide solution for 3 hours. The dark brown solution was cooled and extracted with ether (Extract *a*). The solution was then acidified with hydrochloric acid and again extracted with ether (Extract *b*). The extract (*a*) furnished a small quantity of an oil which gave a semicarbazone, m.p. 191-92°, undepressed on admixture with a specimen of acetophenone semicarbazone, m.p. 192-94°.

The residue from *Extract* (*b*) was twice sublimed in vacuum and proved to be benzoic acid by m.p. and mixed m.p.

Methylation of Baicalein.—An excess of diazomethane in ether was added to a methyl alcoholic solution of baicalein and kept overnight. The cream-coloured crystalline residue left after the removal of the solvents, was sublimed at 199-200°/0·05 mm. and then crystallised from dilute methyl alcohol, when silky needles, m.p, 160-61°, were obtained. A mixture of this substance and baicalein trimethyl ether melted at 161-62°.

Isolation of 'Oroxylol'.—The filtrates left after the separation of baicalein as the lead salt, was freed from lead by means of sulphuretted

hydrogen and the filtrate concentrated on the water-bath when bright yellow needles, m.p. 218-25°, were obtained; yield nearly 1% calculated on the air-dried bark.

Attempts to Separate the Constituents of 'Oroxylol'.

(*a*) *By Crystalisation.*—Repeated crystallisations from alcohol gave bright yellow needles, m.p. 227-29°. (Found : C, 79·23 ; H, 4·68 ; OMe, 7·79 per cent).

(*b*) *By Fractional Extraction with Chloroform.*—3 G. of 'oroxylol' were successively digested with 10 c.c. of chloroform for half an hour, cooled to room temperature and filtered.

Fraction.	(*a*) M.p. of residue insoluble in chloroform.	(*b*) M.p. of residue obtained from the filtrate.
I	218-32°	Crystals with oil
II	217-20°	173-76°
III	220-26°	,,
IV	226-28°	180-81°
V	224-27°	196-98°
VI	228-30°	204-10°

There was only slight rise in m.p. after the seventh extraction of the residue insoluble in chloroform. Fraction (VI*a*) was analysed. (Found : C, 69·65 ; H, 4·29 ; OMe, 7·52. $C_{16}H_{12}O_5$ requires C, 67·60 ; H, 4·23 ; OMe, 10·92 per cent). 60% 6-methylbaicalein ($C_6H_{12}O_5$) + 40% chrysin, ($C_{15}H_{10}O_4$) requires C, 68·92 ; H, 4·11 ; OMe, 6·54 per cent.

The fractions (II*b*) and (III*b*) were washed with cold benzene and then dissolved in alcohol, treated with a few drops of neutral lead acetate and filtered. The filtrate after removal of lead and concentration gave yellow prism, m p. 222-23°. (Found : C, 68·16 ; H, 4·2 ; OMe, 9·78 per cent. Baicalein-6-methylether requires C, 67·6 ; H, 4·23 ; OMe, 10·92 per cent).

Fractions (IV*b*) and (V*b*) were dissolved in ammonia, filtered and the filtrate fractionally precipitated with dilute hydrochloric acid. The first fraction after crystallisation from alcohol melted at 200-2° (Found : OMe, 8·74%). The second fraction after crystallisation from alcohol melted at 186-88° (Found : OMe, 8·98%). Another portion of the combined fractions (IV*b*) and (V*b*) was sublimed at 0·025 mm. pressure and the fraction obtained below 200° collected. It melted indefinitely from 202° to 211°.

Fraction (VI*a*) was subjected to fractional sublimation at 0·04 mm. pressure. The product obtained at 185-200° (bath temperature) formed deep yellow needles, m.p. 219-22°. Resublimation in high vacuum gave a product which, when crystallised from dilute acetone, melted at 210-214°. The product which sublimed above 200°/0·04 mm. formed pale yellow plates

from alcohol and melted at 269-70°. Further sublimation and recrystallisation gave a substance, m.p. 273-75°, which was identified as chrysin by mixed m.p.

(*c*) *By Fractional Precipitation from Alkaline Solution.*—'Oroxylol' was dissolved in dilute ammonia, allowed to stand for some time and then filtered to remove a little slimy matter. The clear filtrate was treated with dilute hydrochloric acid drop by drop with constant shaking, till it became turbid. It was filtered at this stage and the turbid filtrate was treated with a little dilute hydrochloric acid and the precipitate removed. The filtrate was again treated with a small quantity of hydrochloric acid and the precipitate collected. In this way several fractions were collected ; these were washed with water and dried. They were then separately crystallised from alcohol and their melting points were determined. The fractions melting within 5° of each other were mixed together and recrystallised. The major portion, after final purification, was found to melt at 233-35° (Found : C, 70·13 ; H, 4·63 per cent).

Hydrolysis of 'Oroxylol'.—A portion of the fraction, m.p. 228-30°, was hydrolysed with 10% alkali and the product worked up as in the case of baicalein (*vide supra*). Benzoic acid and acetophenone-semicarbazone were isolated and identified.

Demethyiation of 'Oroxylol'.—A specimen (1 g.) of the fraction, m.p. 227-30°, was gently boiled with 20 c.c. of hydriodic acid (*d* 1·7) and enough phenol to keep the substance in solution for 2 hours. The mixture was then poured into a dilute aqueous solution of sodium sulphite. The greenish yellow precipitate was collected, washed with water, dissolved in warm alcohol and treated with an excess of lead acetate slightly acidified with acetic acid. The greenish brown precipitate was worked up for baicalein (*vide supra*), the yield of which was nearly 0·6 g. It was identified as baicalein in the usual manner.

The filtrate left after the separation of baicalein as the lead salt, was freed from lead in the usual manner and concentrated when pale yellow plates (0·35 g.) were obtained. After crystallisation from alcohol, it melted at 274-75° and did not depress the m. p. of an authentic specimen of chrysin. (Found : C, 70·85 ; H, 4·15. $C_{15}H_{10}O_4$ requires C, 70·87 ; H, 3·94 per cent).

In conclusion we wish to express our thanks to Mr. Sarbakali Banerjee for some preliminary work in connexion with this investigation. Our thanks are also due to Prof. S. Hattori and Dr. K. Venkataraman for some specimens.

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ESTIMATION OF CHROMIUM BY ALAKALINE MERCURIC OXIDE.

By Prodoshchandra Raychaudhury.

It has been found that the tervalent chromium ion is converted into a hexavalent one by the action of alkaline mercuric oxide with the separation of mercury.

Quite a large number of methods have been described in the literature for the oxidation of tervalent Cr^{+++} into a hexavalent one. Action of almost all the well-known oxidising agents has been studied. Even alkaline silver oxide was found by Meneghini (*Gazzetta*, 1912, **42**, 1), to oxidise chromic salt solutions to chromates. He represented the reaction by the following equation

$$2\ Cr(OH)_3 + 6\ Ag_2O + 4\ NaOH = 2Na_2\ CrO_4 + 3\ Ag_4O + 5\ H_2O.$$

But Hans (*Z. anorg. Chem.*, 1924, **140**, 337) and Lyden (*ibid.*, 1937, **234**, 59) have subsequently shown that Ag_2O was reduced to metallic silver and no sub-oxide of the type described by Meneghini was formed.

$$Cr_2O_3 + 3\ Ag_2O = 2\ CrO_3 + 6\ Ag$$

or

$$2\ Cr(OH)_3 + 3\ Ag_2O + 4\ NaOH = 2\ Na_2\ CrO_4 + 6\ Ag + 5\ H_2O.$$

Alkaline murcuric oxide has also been used with success as an oxidising agent in organic chemistry, and methods have been developed for the estimation of reducing sugars and polyhydric alcohols with its help. It was, therefore, considered of some interest to study the action of aikaline mercuric oxide on chromic salt solutions. As a result, it has now been found, as described in the present paper, that chromic salts in solution are completely oxidised to chromate by alkaline mercuric oxide under suitable conditions, thus permitting the estimation of chromium volumetrically in a solution. The method, however, fails in presence of iron. The iron hydroxide precipitate always holds tenaciously quite an appreciable amount of chromium. The reaction proceeds according to the simple equation,

$$3\ HgO + Cr_2O_3 = 2\ CrO_3 + 3Hg$$

or

$$3\ HgO + 2\ Cr(OH)_3 + 4\ NaOH = 2\ Na_2CrO_4 + 3\ Hg + 5H_2O$$

EXPERIMENTAL.

Procedure.—The solution of chromic salt (chloride, sulphate and nitrate), mixed with the requisite quantity of mercuric chloride solution, was treated with the caustic soda solution. The mixture containing the precipitated mercuric oxide in suspension was boiled for about 13 minutes. The yellow HgO turned black or brown due to the seperation of metallic mercury, and the solution became yellow due to the formation of chromate. This was filtered and washed with hot water till free from alkali. Chromium was estimated iodometrically in the filtrate after acidification. The results are incorporated in Table I.

TABLE I.

3G. of $HgCl_2$ require 0·885 g. NaOH for precipitation as HgO.

$HgCl_2$.	Alkali added.	Chromium used.	Chromium found.	$HgCl_2$.	Alkali added.	Chromium used.	Chromium found.
3 g.	1 g.	0·04393 g.	0·002686 g.	0·5 g.	5 g.	0·04125 g.	0·04125 g.
,,	2	,,	0·04246	,,	,,	,,	0·04125
,,	3	,,	0·04298	1·0	6	,,	0·04125
,,	4	,,	0·04307	,,	,,	,,	0·04125
,,	5	,,	0·04316	,,	5	,,	0·04107
4·5	3	,,	0·04263	,,	,,	,,	0·04107
,,	5	,,	0·04281	1·5	7	,,	0·04107
,,	7	,,	0·04307	,,	,,	,,	0·04107
6	4	,,	0·04246 g.	0·75	4	,,	0·04090
,,	10	,,	0·04298	,,	,,	,,	0·04090
,,	5	0·04125	0·04119	1·5	9	,,	0·04125
				,,	,,	,,	0·04125

From the above results it is clear that about six times or more of alkali by weight compared to that of $HgCl_2$ is necessary for complete oxidation. Table II gives results of such quantitative reactions.

TABLE II.

1 G. $HgCl_2$ requires 0·295 g. of NaOH for precipitation as HgO.

$HgCl_2$ used.	Alkali used.	Chromium added.	Chromium found.	$HgCl_2$ used.	Alkali used.	Chromium added.	Chromium found.
0·5 g.	3 g.	0·01161 g.	0·01161 g.	1·5 g.	9 g.	0·03986 g.	0·03986g.
,,	,,	0·01161	0·01161	,,	,,	0·03986	0·03986
,,	,,	0·01716	0·01716	1·0	6	0·04576	0·04576
,,	,,	0·01716	0·01716	,,	,,	0·04576	0.04576
,,	,,	0·02288	0·02288	0·7	,,	0·04783	0·04783
,,	,,	0·02288	0·02288	,,	,,	0·04783	0·04783
,,	5	0·03206	0·03206	1·0	,,	0·05590	0·05590
,,	,,	0·03206	0·03206	,,	,,	0·05590	0·05590

TABLE III.

	Alkali used.	Cr taken.	Cr found.
0·57 G. red HgO	4 g.	0·02288 g.	No reaction.
0·4 G. $HgCl_2$ precipitated with NaOH and freed from NaOH	3	0·01716	0·017156 g.

The results show that red dry HgO, as available in the market, fails to react even in presence of an excess of alkali, while freshly precipitated HgO is quite active,

TABLE IV.

Cr used in each expt. = 0·05384 g., requires 0·02482 g. O_2 for complete oxidation. NaOH used in each expt. = 5g.

$HgCl_2$.	O_2 equiv from $HgCl_2$ used.	Cr from the chromate.	O_2 equiv. from the chromate formed.
0·207254 g.	0·012232 g.	0·02655 g.	0·01226 g.
0·310881	0·018348	0·03992	0·01841
0·414508	0·024464	0·05232	0·02450
0·466322	0·027522	0·05384	0·02482
0·518135	0·030580	0·05384	0·02482

This table shows that the reaction proceeds according to the simple equation,

$$Cr_2O_3 + 3\ HgO = 2\ CrO_3 + 3\ Hg,$$

and no lower oxide of mercury is formed.

It is interesting to note in this connection that the reverse action, *i.e.*, the oxidation of metallic Hg by chromate in acid solution leads to the formation of mercurous oxide or salts, as has been recently shown by Paris (*Bull. Soc. chim.*, 1937, **4**, 1803).

My grateful thanks are due to Prof. P. Rây for his kindly suggesting this piece of work to me and for all the facilities I have received in carrying out this work in his laboratory.

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FORMALDEHYDE FORMATION IN THE PHOTO-OXIDATION OF ORGANIC COMPOUNDS AND THE FORMALDEHYDE THEORY OF CARBON ASSIMILATION.

By Atma Ram and N. R. Dhar.

It has been observed that formaldehyde is produced during the photo-oxidation of organic compounds such as, glycine, citric acid, acetic acid, oxalic acid, canesugar etc.

On the basis of the amount of formaldehyde formed during photo-oxidation, all the organic substances investigated by the authors have been di ided into three groups :—

(*a*) Formaldehyde obtained as a direct product of photo-oxidation such as, glycine, acetic acid, methyl violet etc .

(*b*) Formaldehyde obtained may be partly due to the direct photo-oxidation of the substance and partly due to its photosynthesis from carbon dioxide generated during oxidation.

(*c*) Formaldehyde detected may be due to its photosynthesis from energy-rich carbon dioxide produced during photo-oxidation.

The production of formaldehyde is not only limited to substances of biochemical origin as observed by Moore, Wager etc, but it can be detected during the photo-oxidation of any organic substances provided it can be oxidised to carbon dioxide and water in light.

It is easier to obtain formaldehyde from carbon dioxide generated during the photo-oxidation from organic compounds than from potassium bicarbonate solutions or carbon dioxide solution because the former is associated with energy evolved during oxidation.

The energy produced during respiration is concluded to supply a part of the energy need for photosynthesis.

Besides light, carbon dioxide, moisture and chlorophyll, energy from respiration seems also necessary for photosynthesis.

Numerous attempts have been made to imitate *in vitro* the phenomenon of photosynthesis in plants by exposing solutions of carbonic acid or bicarbonate with or without photocatalysts to different radiations. In the natural process, however, the carbon assimilation, which is a highly endothermal reaction

$$(6\,CO_2 + 6\,H_2O + 676\ KCal = C_6H_{12}O_6 + 6O_2),$$

is influenced not only by the light radiations but is also aided by the plant respiration.

It is well known that in the process of germination of seeds, there is considerable loss of energy-rich material by oxidation and this phenomenon supplies the major portion of the energy required for the seedling. It appears that under natural conditions, the endothermal reaction involved

in formaldehyde or glucose formation in plants takes place by absorption of solar radiations and by the partial utilisation of the energy of respiration. Hence, it seems likely that it is easier to obtain formaldehyde or any other energy-rich compound form carbonic acid and bicarbonate solutions on exposure to light when a suitable exothermal reaction is also taking place in the system along with the photosynthetic reaction.

The researches of Palit and Dhar (*cf.* Dhar, "New Conceptions in Biochemistry", 1932) show that sugars and other energy materials are oxidised by air when their solutions or suspensions are exposed to light and air. The carbonic acid formed in these oxidations not only receives the light radiations but is also supplied with the energy liberated in the oxidations of the sugars and other carbonaceous compounds. Hence, the photoformation of formaldehyde is likely to be easier in such cases, where two energy sources are available than in those in which carbonic acid or bicarbonate solutions are exposed to light alone. Moreover, it has been discovered in this laboratory that sunlight or artificial light markedly increases nitrogen fixation ($N_2+O_2+43{\cdot}2$ K.Cal$=2NO$) in soils mixed with energy materials. In this case, the endothermal reaction involved in nitrogen fixation is not only aided by the energy of the caboyhydrate oxidation ($C_6H_{12}O_6+6O_2=6CO_2+6H_2O+676$ K.Cal) but is also accilerated by the absorption of light radiations, which are actually utilised in bringing about more nitrogen fixation. The nitrogen fixation in light, where both the oxidation energy and the radiation energy are utilised, is nearly twice that of the fixation in the dark, when only the energy of the oxidation is available for the endothermal reaction involved in nitrogen fixation.

We have carried on a systematic work on the photoformation of formaldehyde by the photo-oxidation of different organic substances for several years and most of our results are recorded in this paper, which is a continuation of a previous publication (Dhar and Atma Ram, *J. Indian Chem. Soc.*, 1933, **10**, 287) in which a few observations were reported.

EXPERIMENTAL.

Merck's or Kahlbaum's samples of the organic substances were used and were further purified by crystallisation. Dilute solutions usually $M/100$ or $M/1000$ in the case of the dyes were exposed to bright sunshine in Jena flasks, well stoppered and kept in a water-bath at a constant temperature. After an exposure of six hours or more, an aliquot part from the exposed solutions was distilled and the presence of formaldehyde was detected by Schryver's test. When it was established that formaldehyde

is actually produced during the photo-oxidation of a certain substance, it was necessary to estimate this amount.

The estimation of very small quantities of formaldehyde is difficult. The methods for its estimation already in vogue, such as, the iodometric and the urotropin method were both tried with unsatisfactory results. We have, therefore, applied Schryver's test for a colorimetric estimation of formaldehyde, which is carried on as follows. It may be pointed out here that it is possible to detect formaldehyde when present even in such small amounts as 0·000015 g. in 100 c. c. provided all the precautions are taken.

Estimation of Formaldehyde by the Colorimetric Method.

All the reagents (phenylhydrazine hydrochloride and potassium ferricyanide) used in the test should be prepared fresh. A standard solution of formaldehyde was prepared by diluting solution whose strength had already been determined by the iodometric method. 5 C. c. of the solution to be estimated were taken in a clear test tube and 5 c.c. of the standard solution in another one, and the reagents added in the proper order (phenylhydrazine, ferricyanide and hydrochoric acid). When the colour developed, which usually takes about five minutes, the contents of the two tubes were poured into those of the colorimeter, and the colours of the two tubes matched.

Group I.

Formaldehyde produced as a direct product of photo-oxidation.

TABLE I.

50 C·c. solutions in water exposed at 30° for 8 hours in open beakers.

Substances exposed.	Amount of H·CHO produced per 100 c.c. soln.	Substances exposed.	Amount of 4 CHO produced per 100 c.c. soln.
		Acids.	
2*N*-Acetic acid	0·0011 g.	*N*/5-Malic acid	0·001 g.
N/5-Citric acid	0.0012	*N*/5-Monochloroacetic acid	0·00095
N/5-Lactic acid	0·0009	*N*/100-Glycine	0·0013
		Dyes.	
0·02%-Malachite green	0·00096	0·02%-Gentian violet	0·00085
0·02%-Methylene blue	0·00075	0·02%-Auramine	0·0007
0·02%-Methyl violet	0·0012	0·02%-Victoria blue	0·00091
0·02%-Crystal violet	0·0009	0·02%-Acridine orange	0·00072
		Miscellaneous.	
1%-Guaiacol	0·0011	2%-Glycol	0·00086
2%-Glycerol	0·0009	Suspension containing 0·02 g. chlorophyll	0·00096

Group II.

TABLE II.

Conditions same as in Table I.

Substance exposed.	Amount of H·CHO formed in g. per 100 c. c. of the soln.	Substance exposed.	Amount of H·CHO formed in g. per 100 c.c. of the soln.
N/5-Tartaric acid	0·0002	0·02% Gallomine blue	0·00045
N/1-Propionic acid	0·00021	0·02% Aurine	0·00025
N/1-Butyric acid	0·00016	1% Galactose	0·00036
N/5-Pyruvic acid	0·00034	1% fructose (50 c.c.)	0·00052
0·02%-Phloxine	0·00041		

Group III.

Formaldehyde produced as a result of its photosynthesis from the carbon dioxide produced during the photo-oxidation.

TABLE III.

Conditions same as in Table I.

Substance exposed.	Amount of H·CHO formed in g. per 100 c. c. of the soln.	Substance exposed.	Amount of H·CHO formed in g. per 100 c.c. of the soln.
		Carbohydrates.	
1% Cane sugar	0·00005	1% Mannitol	0·000055
Glucose	0·000045	Sorbitol	0·00003
Arabinose	0·00004	Starch	0·00003
		Inulin	0·000035
		Acids	
N/5-Oxalic acid	0·000065	N/5-glutaric acid	0·00004
Malonic acid	0·000052	adepic acid	0·000035
Succinic acid	0·00005		

TABLE III. (*contd*).

Amino-acids.

N/50-Alanine (a)	0·00007	*N*/50-Cystein	0·00006
Phenylalanine	0·00004	Cysteine	0·00009
Leucine	0·000055	Tryptophan	0·000085
Valine	0·00004	Arginine	0·00015
Lysine	0·000048	Tyrosine (a)	0·000074
Aspartic acid	0·000074	Hippuric acid	0·000042
Glutamic acid	0·000067	Histidine	0·00004
Asparagine	0·000045	Uric acid	0·00003

Salts and Esters.

N/1-Sodium acetate	0·000093	*N*/100-Sodium oleate	0·000098
Sodium formate	0·000075	Sodium stearate	0·000084
Sodium propionate	0·000067	Sodium palmitate	0·000078
Sodium butyrate	0·000054	1%-Ethyl acetate	0·000055
Sodium oxalate	0·000063		

Dyes.

0·02%-Congo red	0·00003	0·02%-Methyl red	0·00004
Safranine	0·000056	Naphthachrome	0·000013
Acridine yellow	0·00006	Pinaverdol	0·00003
Orthochrome	0·00015	Benzopurpurin	0·00004
Pinachrome	0·00009	Chrysophenine	0·000012
Pinaflavol	0·0001	Brilliant green	0·00007
Pinacyanole	0·00008	Neutral red	0·0001
Rhodamin	0·00006	Aniline orange	0·00008
Eosin	0·000012	Bixin	0·00011
		Aniline green	0·00008

Miscellaneous.

0·3%-Gelatine	0·000065	Dilute suspension of ergosterol	0·000065
0·5%-Creatinine	0·00007	Suspension of chloesterol	0·000075
0·5%-Creatin	0·000054		

Oxygen is necessary for the Production of Formaldehyde from Organic Substances in Light.

In a previous communication (Dhar and Atma Ram, *loc. cit.*) the production of formaldehyde from substances of the first group has been explained as a direct product of photo-oxidation. If that is so, oxygen should be necessary in order that any formaldehyde may be formed from glycine, citric acid etc. Experiments were carried out to this point, by exposing dilute solutions of glycine and citric acid to sunlight in presence of oxygen and also in atmospheres of nitrogen, hydrogen, carbon dioxide etc. The following table contains some of the results.

TABLE IV.

50 C. c. of solution exposed in soft glass bulbs for 8 hours at 30°.

Gas with which the soln. has been saturated.	Amount of H.CHO formed per 100 c.c. of the soln.	
	N/50-Glycine.	*N*/5-Citric acid.
Oxgyen	0·0011 g.	0·0012 g.
Nitrogen	0·00005	0·000045
Carbon dioxide	0·000065	0·00007
Hydrogen	0·00003	0·00003

In all cases conductivity water saturated with the different gases was also exposed in glass bulbs and tested with the exposed solutions. The absence of formaldehyde in all these bulbs and also in the dark, points out that formaldehyde in the above experiments is not obtained from impurities, but is produced from the organic substance undergoing photo-oxidation.

From these results it will be seen that the production of formaldehyde from glycine or citric acid involves a chemical change in which oxygen plays a very important part. It appears that the generation of formaldehyde from substances of the first group is a case of photo-oxidation and not of photodecomposition, *i. e.*, light and air or oxygen are both necessary for this reaction.

Ratio of the Number of Molecules of the Organic Sustances oxidised to those of Formaldehyde formed.

In order to throw light on the problem whether formaldehyde obtained from the photo-oxidation of the substances of the first group is a direct

product of the photo-oxidation, experiments were carried on for determining the ratio of the number of molecules of the organic substances oxidised to those of formaldehyde formed. We have advanced the view that the important chemical change involved in the photo-oxidation of glycine can be represented as follows :—

$$NH_2 \cdot CH_2\ COOH + (O) = CO_2 + NH_3 + H \cdot CHO$$

From this equation, it is evident that a molecule of glycine on its photo-oxidation gives rise to one molecule each of ammonia and formaldehyde. If the amount of glycine oxidised and that of formaldehyde formed can be estimated, this ratio can be easily calculated. The amount of amino-acid oxidised by air in light has been determined by treating it before and after the experiment with potassium permanganate and sodium carbonate and estimating the amount of ammonia produced. From the difference the amount of amino-acid oxidised by air in light can be calculated.

As a result of their researches on the photo-decomposition of formaldehyde, Norrish and Kirkbridge (*J. Chem. Soc.*, 1932, 1518) have observed that the main products of decomposition are carbon monoxide and hydrogen. It seems likely that formaldehyde produced in our oxidation experiments may be partially lost in this way. Thus in calculating the ratio, a correction has to be made for the amount of formaldehyde lost which was determined by a separate experiment.

The following results have been obtained on the ratio : NH_2CH_2COOH/HCHO, making an allowance for the amount of formaldehyde decomposed. The amounts of amino-acids oxidised and the formaldehyde formed, have been expressed in g. moles.

TABLE V.

M/100-Solution (50 c.c.) exposed to sunlight for 8 hours at 30°.

	Amino-acid oxidised in g. moles/100 c.c.	HCHO detected in g. moles/100 c.c.	HCHO that would have been detected without decomp.	Ratio with decomposition.	Ratio without decomposition.
I.	0·00002	0·000016	0·0000185	1·1	1·2
II.	0·000019	0·000017	0·0000175	1·06	1·2
III.	0·000021	0·000017	0.0000185	1·14	1·24

From the foregoing results it appears that formaldehyde produced during the photo-oxidation of glycine is a direct product of the photo-oxidation reaction, as the ratio of the amino-acid oxidised to the formaldehyde formed is nearly unity.

Influence of the Wave-length of the Light on the Photoformation of Formaldehyde from Substances of the First Group.

In order to study the influence of different wave-lengths of light on these reactions, comparative experiments were performed by exposing dilute solutions of glycine, citric acid, malic acid etc., in quartz flasks. It has generally been found that the yield of formaldehyde from these substances is increased in ultraviolet light. The following table contains some of the results.

TABLE VI.

50 C. c. of the solution exposed for 6 hours at 30°.

Substance exposed.	Amount of formaldehyde per 100 c.c. Total light from	
	1000 watt gas filled tungsten lamp.	mercury vapour lamp.
N/100-Glycine	0·00025 g.	0·00045 g.
N/10-Citric acid	0·00035	0·0006
N/10-Malic acid	0·0002	0·00046

It is seen, therefore, that ultraviolet light has a greater effect on the yield of formaldehyde from glycine, citric acid etc., than the total light from a gasfilled tungsten filament lamp.

Temperature Coefficient of the Photoformation of Formaldehyde from Substances of the First Group.

During recent years, a systematic study of the influence of temperature on photochemical reactions has been helpful in the elucidation of the mechanism of such processes. With this object in view, an attempt was made to study the temperature coefficients of these reactions. It may be stated that in this investigation the temperature coefficient means the ratio of the yields of formaldehyde at two different temperatures. This is due to the fact that the velocity of the photo-oxidation is too slow for the measurement of the actual velocity coefficient.

$N/50$. solution of glycine and 0·01% solution of methyl violet were exposed in 500 c. c. Jena glass flasks well stoppered and kept at different temperatures. In the following tables some of the results have been summarised.

TABLE VII.

$N/50$-Solution glycine (50 c.c.) exposed for 6 hours.

Temp.	Amount of HCHO. formed per 100 c.c.	Temp. coeff.
20°	0·00018 g.	
		1·66
30°	0·0003	
		1·4
40°	0·00042	
		1·25
50°	0·00052	
		1·06
60°	0·00055	

TABLE VIII.

Methyl violet (0·01%).

Temp.	Amount of H·CHO formed per 100 c c.	Amount of dye decomposed per 100 c.c.	Temp. coeff.
30°	0·0000079 g. moles.	0·0000027 g. moles.	
			1·2
40°	0·0000095	0·0000035	
			1·1
50°	0·0000105	0·0000043	

An examination of these results, shows that the temperature coefficient of the photoformation of formaldehyde from substances of the first group is somewhat more than unity and goes on decreasing as the temperature rises. These are cases of photo-oxidations, since no formaldehyde is produced in the dark.

It has already been pointed out that the origin of formaldehyde, detected during the photo-oxidation of substances like oxalic acid, α-alanine, cane sugar etc., is its photosynthesis from carbon dioxide and water, which are produced during the photo-oxidation of organic compounds. From comparative experiments it has been found that a solution of α-alanine is oxidised to a greater extent than a solution of glycine of the same concentration, although the amount of formaldehyde formed from the latter is about twenty times greater that that obtained from α-alanine. This is probably

due to the fact that formaldehyde is obtained from glycine as a direct product of photo-oxidation of the latter, whilst from α-alanine it is produced as a result of its photosynthesis from the energy-rich carbon dioxide and water generated during photo-oxidation. In order to understand the mechanism of the formation of formaldehyde from substances of the third group, comparative experiments on the ratio of the amount of the substance oxidised to that of formaldehyde formed have been carried out. Since oxalic, malonic, succinic acids, etc. can be easily estimated by titrating against a standard baryta solution in the usual manner by using phenolphthalein as an indicator, it was considered advisable to study the photo-oxidation of these acids. Moreover, these experiments have also been carried out with amino-acids like α-alanine, valine, leucine, aspartic acid etc.

Experimental Determination of the Ratio of the Amount of Organic Substance Photo-oxidised to the Amount of Formaldehyde formed from Substances of the Third Group.

The amount of formaldehyde produced from oxalic acid, α-alanine etc. being small, it is necessary that the amount of formaldehyde decomposed on exposure to light should be accounted for in determining the ratio of the acid decomposed to formaldehyde formed.

Standard solutions of the three acids were exposed in quartz flasks of the same capacity, well fitted with stoppers. The amount of the *acid oxidised* was estimated by titrating it against a standard baryta solution in the usual manner with phenolphthalein as an indicator, before and after the exposure. The amount of formaldehyde was determined by the colorimetric method. The amount of the substances oxidised and the formaldehyde formed have been expressed in g. moles in these tables.

TABLE IX.

50 C.c. of $N/5$-solution exposed for 8 hours at 30°.

Substance exposed.	Amount of H·CHO formed per 100 c.c.	Amount of the acid oxidised.		Ratio $\frac{\text{Acid oxidised.}}{\text{H·CHO}}$
Oxalic acid	0·0000033 g. moles	0·00109 g. moles	5·4%	330
Malonic	0·0000027	0·00033	1·6	122
Succinic	0·0000023	0·0002	1·0	89
Glutaric	0·0000018	0·00015	0·75	83

It will be seen from these observations, that as many as 330 molecules of oxalic acid are oxidised per molecule of formaldehyde formed. Moreover, in the homologous series of the dicarboxylic acids. the amount of formaldehyde produced from a solution of oxalic acid is greatest and goes on decreasing as the molecular weight of the homologue increases. But the percentage of acid oxidised to that of formaldehyde formed goes on decreasing with increasing molecular weights.

TABLE X.

Substance exposed.	Amount of H·CHO formed per 100 c c.	Amount of the substance oxidised per 100 c.c.		Ratio $\frac{\text{Substance oxidised.}}{\text{H·CHO}}$
Cane sugar	0·0000017 g. moles	0·00015 g. moles	1·5%	88
Glucose	0·0000017	0·00016	1·6	94
α-Alanine	0·0000018	0·00021	2·1	117
Aspartic acid	0·0000015	0·00018	1·8	120

These results lend support to the view that formaldehyde produced from substances of the third group owes its origin to photosynthesis from carbon dioxide and water produced during photo-oxidation, as the ratio of the amount of substance oxidised to the amount of formaldehyde formed is much higher than unity as obtained with substances of the first group.

Comparative Experiments on the Ratio of the Amount of Substance oxidised to that of Formaldehyde formed from Substances of Different Groups.

It has been stated before that the production of formaldehyde from substance of the third group is due to its photosynthesis from the energy-rich carbon dioxide generated during photo-oxidation. If that is true, the ratio of the amount of the substance oxidised to amount of formaldehyde formed, should be greater with substances of the third group than with those of the first group, where it is obtained as a direct product of the photo-oxidation. The following experiments were carried on by exposing aqueous solutions of different substances having the same concentration.

TABLE XI.

$M/100$ solution (50 c.c.) exposed in quartz flasks for 8 hours at 30°.

Group No	Substance.	Amount of H·CHO formed per 100 c.c.	Amount of the acid oxidised per 100 c c.	Ratio.
I	Glycine	0·00003 g. moles	0·000033 g. moles	1·1
II	α-Alanine	0·0000017	0·00012	71
III	Valine	0·0000012	0·000071	59

It is evident from the results that the ratio of the amount of substance oxidised to that of formaldehyde formed is greater with the third group of substances than with those of the first group. The amount of formaldehyde produced from a substance of the third group is much less than that obtained from a substance having the same chemical character but belonging to the first group. On the other hand, the percentage amount of the substance oxidised of the third group is much greater than that of a compound belonging to the first group.

This observation is rather striking and appears to lead to the conclusion that the generation of formaldehyde from substances of the third group cannot be a case of direct photo-oxidation, since it is not likely that the direct formation of a molecule of formaldehyde is caused by the oxidation of three hundred molecules of oxalic acid or sugar. The mechanism of the photoformation of formaldehyde from oxalic acid, cane sugar etc. seems to be its photosynthesis from the energy-rich carbon dioxide which is a product of photo-oxidation.

Temperature Coefficient of Photosynthesis of Formaldehyde from Energy-rich Carbon Dioxide produced during Photo-oxidation of Organic Substances.

In preceding pages, it has been pointed out that the temperature coefficient of photoformation of formaldehyde from substances of the first group goes on decreasing as the temperature increases and tends to be unity at about 60°. For instance, in the case of glycine the temperature coefficient between 10° and 20° is 1·8 and between 50°-60° is 1·06. Since almost all the oxidation reactions are accelerated by increase of temperature, it is expected that the production of formaldehyde from substances of the third group may also be increased with temperature, if it is a case of direct photo-oxidation. Hence an investigation of the temperature coefficient of these

reactions may also throw light on the generation of formaldehyde in the third group of substances. In order to test this point, a systematic study of the temperature coefficient of these reactions was undertaken.

Dilute solutions of oxalic acid, malonic acid, orthochrome etc. were exposed in thin layers in petri dishes and covered by quartz covers, thereby allowing all the light to fall on the exposed solutions and avoiding the dust. Since it is necessary to show that no formaldehyde is obtained in these experiments due to contamination, blank experiments were carried on. Solutions of substances under investigation were kept in shade in the same locality contained in similar vessels. In a clean dish some distilled water was exposed side by side. The temperature of the exposed solutions was maintained within 1°.

TABLE XII.

Oxalic acid.

N/5-Solution (50 c. c.) exposed in petri dishes covered with quartz cover for 8 hours.

Temp.	Amount of the		Temp. coeff. with regard to	
	acid oxidised per 100 c.c.	H·CHO formed per 100 c. c.	acid oxidised.	H·CHO formed.
10°	0·0003 g. moles	0·000001 g. moles	2·0	1·9
20°	0·0006	0·0000019	1·5	1·5
30°	0·0009	0·000003	1·3	0·76
40°	0·00115	0·0000023	1·17	0·6
50°	0·00133	0·0000014		

These results are interesting from the view-point of the mechanism of the photoformation of formaldehyde from substances like oxalic acid. On an examination of columns 4 and 5, it will be seen that up to 30° the temperature coefficient of the reaction is more than unity with regard to formaldehyde formation as well as acid oxidised. On the other hand, a peculiar behaviour is observed if these experiments are carried on at temperatures above 30°. The value of the temperature coefficient with regard to formaldehyde formation becomes less than unity, while with regard to acid decomposition it still remains more than unity.

TABLE XIII.

Orthochrome.

0·02% Solution (50 c. c.) exposed in petri dishes covered with quartz covers for 8 hours.

Temp.	Amount of the		Temp. coefficient with regard to	
	HCHO formed per 100 c c.	dye decomposed per 100 c.c.	HCHO formation.	dye decomposition.
10°	0·0000007 g. moles	0·000028 g moles	2·1	2·2
20°	0·0000015	0·00006	1·6	1·7
30°	0·0000025	0·0001	0·6	1·3
40°	0 0000015	0·00013	0·53	1·15
50°	0·0000008	0·00015		

These results are also in agreement with those obtained with oxalic acid. The yield of formaldehyde goes on increasing upto 30° but becomes less as the temperature is raised higher than 30°. It seems that this influence of temperature on the photoformation of formaldehyde from oxalic acid, orthochrome etc. is a general characteristic of the third group of compounds.

Comparison of Temperature Coefficient of the Oxidation of Substances of the Groups I and III.

In the following tables temperature coefficients of the two classes have been compared taking citric acid, oxalic acid, methyl violet and orthochrome as typical examples.

TABLE XIV.

Acids.

Temp. limit.	Temperature coefficient with regard to			
	formaldehyde formation.		acid decomposition.	
	Citric acid.	Oxalic acid.	Citric acid	Oxalic acid.
20-30°	1·7	1·5	1·77	1·5
30-40°	1·49	0·76	1 5	1·3
40-50°	1·18	0·6	1·3	1 17

TABLE XV

Dyestuffs.

Temp limit.	Temperature coefficient with regard to formaldehyde formation		Temperature coefficient with regard to the oxidation of the dye.	
	Methyl violet	Ortho-chrome.	Methyl violet.	Ortho-chrome.
20-30°	1·5	1·6	1·56	1·7
30-40°	1·2	0·6	1·3	1·4
40-50°	1·1	0·53	1·16	1·15

The bearing of these results on the mechanism of the two types of reactions is important. It appears that there is a fundamental difference in the mode of formation of formaldehyde from substances of the first group (citric acid and methyl violet) and the third group (oxalic acid and orthochrome). The temperature coefficient for the first group is more than unity at lower temperatures and tends to unity at higher temperature intervals both with regard to acid and dye decomposition and the formation of formaldehyde.

The effect of temperature on substances of the third group is similar to that on those of the first group, only when the temperature coefficient is calculated with regard to the oxidation of the substance. On the other hand, if it is calculated with regard to photoformation of formaldehyde, the values remain more than unity up to 30°, but decrease rapidly at higher temperatures. The temperature coefficient for substances of the first group, both with regard to formaldehyde formation and the substance oxidised, happens to be of the same order, because here it is formed as a direct product of photo-oxidation. It may seem that these observations suggest that the production of formaldehyde from oxalic acid, orthochrome or in general from those of the third group involves two stages.

(*i*) Photo-oxidation of the organic substance and consequent production of energy-rich carbon dioxide.

(*ii*) Photosynthesis of formaldehyde from energy-rich carbon dioxide and water so produced.

Low yields of formaldehyde at high temperatures may be due to its loss on account of its photodecomposition, which is more prominent at high temperatures ; that is why there is difference between the values of temperature coefficient with the two methods of calculation.

It will be interesting to note here that the temperature coefficient of photosynthesis as observed in plants is less than unity above 30°.

Relation between the Amount of Energy produced during Photo-oxidation of an Organic Substance and the Amount of Formaldehyde formed Photosynthetically.

It is well known that the amount of heat generated per gramme of fat oxidised is 9 calories, whilst with both proteins and carbohydrates it is about 4·1 calories per gramme. In order to find out whether there is any relation between the amount of energy generated in the oxidation of different energy materials and that of formaldehyde photosynthesised during photo-oxidation of an organic substance, we have carried on comparative experiments on the photoformation of formaldehyde not only from the oxidation of organic compounds but have also compared the amount of former obtained by exposing solution of potassium bicarbonate to sunlight. In the following table some of these results have been summarised.

TABLE XVI.

Strength of the solutions exposed = $M/100$ (organic compounds). Strength of the potassium bicarbonate solution = $M/5$. Volume of the solutions exposed = 50 c.c. Containers, quartz flasks. Exposure = 7 hours.

System exposed.	Amount of the substance decomposed or oxidised per 100 c.c.	% of the substance oxidised or decomposed.	Amount of H·CHO formed per 100 c.c.	Ratio of the amount of substance oxidised to that of H·CHO.
$KHCO_3$	0·0098 g. mole	49	0·000001 g. mol.	9800
Sodium oleate	0·00011	1·1	0·0000037	29·9
Sodium palmitate	0·000091	0·9	0·000003	30·3
Sodium stearate	0·000082	0·82	0·0000023	36
Cane sugar	0·00015	1·5	0·0000018	83
Glucose	0·00016	1·6	0·0000017	94
α-Alanine	0·00021	2·1	0·0000018	117
Aspartic acid	0·00018	1·8	0·0000015	120

These results are interesting because they show that the amount of formaldehyde formed by exposing potassium bicarbonate solutions to sunlight in quartz vessels is much smaller than that photosynthesised during photo oxidation of organic substances. Moreover, when we compared the amount

of formaldehyde formed with that of bicarbonate decomposed or the organic substance oxidised, there is a great divergence. The ratio is much greater with the bicarbonates in comparison with the organic compounds. In other words, it is much easier to obtain formaldehyde from carbonic acid generated during the photo-oxidation of an organic compound than from that produced in the decomposition of potassium bicarbonate solutions exposed to light. This is certainly due to the fact that the oxidation of organic matter in air and light generates energy and this is utilised in the photosynthesis of formaldehyde. These observations also show that the amount of formaldehyde obtained is greatest with those substances which generate the maximum amount of energy on their oxidation, *i.e.*, the photoformation of formaldehyde is greater with sodium stearate than with the carbohydrates and amino-acids under identical conditions. It appears that the amount of formaldehyde formed in the photo-oxidation of substances of the third group is directly connected with the energy of oxidation, which is actually utilised in this process along with the radiation energy.

It is concluded, therefore, that the energy generated during oxidation supplies a part of the energy needed for the photosynthesis of formaldehyde. These reactions in which both radiation energy and energy derived from oxidation are utilised in the production of energy-rich compounds appear to be of the same type as in photosynthesis in plants where both the energy of respiration and radiation energy are used up in the synthesis of carbohydrates and other energy materials.

DISCUSSIO

In the preceding pages, it has been shown that formaldehyde can be obtained by exposing aqueous solutions and suspensions of organic substances to light and air. Though the production of formaldehyde from substances of biochemical origin, when their solutions are exposed to light, has been reported by many observers, the origin of its formation has not yet been explained satisfactorily. We have made the interesting observation that the generation of formaldehyde is not only limited to substances of a biochemical nature, but almost any organic substance can produce it when exposed to light and air provided it can undergo photo-oxidation.

Why is it that formaldehyde is produced during the photo-oxidation of organic substances ? It may not be obtained as a direct product of oxidation from all organic compounds. It may be so only in a few cases as in the compounds of the first group.

It seems that the first stage in carbon assimilation taking place *in vitro* and possibly in *vivo* is the photolysis of water molecules into H and OH. Based on this view, we have explained the photoformation of formaldehyde from substances of the third group in the following way

$$H_2O + 112 \text{ K.Cal } (Light) = H + OH.$$
$$\rightarrow$$

The energy required for this operation is supplied by the solar radiations as well as the energy obtained from the oxidation of the organic substances. This energy (112 K.Cal) corresponds to radiations of wave-length 2552Å.

Franck (*Trans. Faraday Soc.*, 1925, **21**, 536) has postulated that complete light absorption by molecules is associated with their breaking up into atoms. Henri ("Strukture des Molekules", Chapter V, 1925) has observed that increase of temperature causes the light absorption by molecules to begin at longer wave-lengths. Dhar and Bhattacharya (*J. Indian Chem. Soc.*, 1934, **11**, 33) have shown that in general an increase in the light absorption by a molecule is associated with its increased chemical reactivity and weakening of the binding forces of the molecules. Hence when a molecule becomes more reactive, it is likely to absorb light more markedly than otherwise.

So far weakening of the binding forces of a molecule and its increased light absorption have been observed under the influeuce of physical agencies like radiation or increase of temperature, but the recent researches carried on in these laboratories, have shown for the first time that chemical agencies can also cause weakening of the binding forces followed by increased light absorption by reacting molecules. Thus in the presence of reducing agents like hydrogen, oxalic acid, oxalates, ferrous sulphate etc., the light absorption and reactivity of the halogen molecules are appreciably increased. It has also been observed that there is more absorption of light by solutions of glycine, acetic acid etc., in presence of oxygen than in its absence.

The increased absorption of light by glycine in presence of oxygen seems to be due to the activation of dissolved oxygen molecules by the presence of those of glycine or acetic acid. It appears very likely, therefore, that in presence of glycine or acetic acid, the reactivity of oxygen will be increased and hence the photo-oxidation of organic substances like glycine and acetic acid by oxygen is possible even in sunlight.

In the second group, the amount of formaldehyde formed during the photo-oxidation is less than that produced in the first class and more than that obtained with the third class of substances. It is difficult to state

definitely whether with the second group of substances formaldehyde is a direct product of photo-oxidation or is formed photochemically from carbon dioxide and water obtained during photo-oxidation of the organic substance.

We have shown that formaldehyde can be obtained from the photochemical reduction of carbon dioxide in aqueous solutions in the presence of photosensitisers. Moreover, formaldehyde is also produced in small amounts from aqueous solutions of potassium or sodium bicarbonate when exposed to light even without a photocatalyst. The reaction,

$$CO_2 + H_2O = H{\cdot}CHO + O_2 - 112, K. \text{ Cal}$$

is highly endothermal and in the absence of an efficient photocatalyst it is difficult to obtain formaldehyde from the photochemical reduction of carbonic acid. Dilute solutions of alkali bicarbonate decompose according to the following equation

$$2KHCO_3 = K_2CO_3 + CO_2 + H_2O$$

with liberation of small amounts of energy. In presence of light, this carbon dioxide may be slightly transformed into formaldehyde by the absorption of more energy. As the energy available from the decomposition of the bicarbonate is small, the amount of formaldehyde produced in this way is exceedingly small.

It is well known that practically all the organic compounds produce carbon dioxide and water when they are slowly oxidised and that this process is accompanied by the generation of energy. For instance, in the case of cane sugar and α alanine, the following reaction may be supposed to take place

$$C_{12}H_{22}O_{11} + 6O_2 = 12\ CO_2 + 11\ H_2O + E,$$

$$CH_3CH{\cdot}NH_2COOH + 3O_2 = NH_3 + 2H_2O + 3CO_2 + E_a$$

where E, and E_a are the amounts of energy generated per g. mol. of cane sugar and α-alanine oxidised respectively. Since carbon dioxide, which is produced in these photo-oxidations is energy-rich, it should be more reactive than a molecule of ordinary carbon dioxide. A molecule of carbon dioxide, which is already in a reactive state, will require less energy to be activated in order to be reduced to formaldehyde than one of ordinary carbon dioxide. It will be interesting to note here that an active variety of carbon dioxide has recently been described by

Fowler (*Proc. Roy. Soc.*, 1933, **A 142**, 362) in which an after-glow has also been observed.

In recent years, several authors notably Haber and Wansborough-Jones, (*Z physikal. Chem.*, 1932, **18B**, 103) have postulated that in many photochemical reactions taking place in aqueous solutions, the primary change is the photolysis of water moleculues into H and OH. It appears that the photolysis of the activated water molecules into H and OH on the leaf surface due to the absorption of light by chlorophyll resembles the cases of photosensitised decomposition of water investigated *in vitro*. The hydrogen atoms so formed may reduce carbon dioxide and the OH radicals may unite to form hydrogen peroxide, which in its turn decomposes into water and oxygen with the liberation of energy as shown by Bonhoeffer and Pearson (*Z. physikal. Chem.*, 1931, **14B**, 1)

$$OH + OH = H_2O + O + 14 \text{ K. Cal.}$$

This may be the probable mechanism of the evolution of oxygen during photosynthesis. The energy given out in the latter reaction may again be used.

Thus formaldehyde which is detected during the photo-oxidation of many organic substances is not always produced as a direct product of oxidation, but is usually obtained due to its photosynthesis from energy-rich carbon dioxide generated during oxidation. It may be mentioned here that we (*J. Phys. Chem.*, 1932, **36**, 567) have been able to obtain traces of formaldehyde from nascent carbon dioxide formed by the interaction of carbonates of barium and strontium with hydrochloric acid, in the absence of photocatalysts.

It will be interesting to note here that Lal (*Ann. Bot.*, 1934, **48**, 273) has observed the formation of alcohol and aldehydes during respiration of apples. These substances are supposed to be produced as a result of their photosynthesis from carbon dioxide generated during respiration in light. It seems probable, therefore, that the energy generated during the photo-oxidation of organic compounds supplies a part of the energy required for the photosyntheis of formaldehyde.

We are of the opinion that the production of formaldehyde from organic substanees when exposed to air and light, which has been regarded as one of the drawbacks of the formaldehyde theory of photosynthesis, may not be quite correct. On the other hand, it is probably one of the strong arguments in favour of this view.

The view that formaldehyde detected in the photo-oxidation of substances of the third group, is produced as a result of its photosynthesis from energy rich carbon dioxide is supported by the following considerations.

(*i*) *Formaldehyde is detected in the photo-oxidation of numerous organic substances.* In the preceding pages, it has been shown that formaldehyde can be detected in the photo-oxidation of substances like oxalic acid, α-alanine, cane sugar, sodium oleate etc. This is not explicable from the view that it may be a direct product of the oxidation.

It is well known that carbon dioxide and water vapour are generally produced during the slow oxidation of organic compounds. The former at the moment of their generation are energy-rich and can readily produce formaldehyde photosynthetically. That is why formaldehyde has been detected in the photo-oxidation of organic substances.

(*ii*) *The ratio of the amount of organic substance photo-oxidised to that of formaldehyde formed is always very high.* Since the efficiency of the photosynthetic process that is, photosynthesis of formaldehyde from carbon dioxide and water is low, it is expected from the mechanism given here, that this ratio should be high. This has already been verified in the case of substances belonging to third group. Whenever formaldehyde is produced as a direct product of photo-oxidation of the organic substance, this ratio is about unity as shown with glycine, acetic acid, methyl violet etc. It will be interesting to note here that with potassium bicarbonate, the ratio of the amount of bicarbonate decomposed to that of formaldehyde formed is as high as 9800. This is because all the molecules of carbon dioxide produced during photo-oxidation or decomposition are not converted into formaldehyde.

(*iii*) *Temperature coefficient of the photoformation of formaldehyde from organic substance of the third group is less than unity above* 30°. An examination of the results summarised in Tables XII, XIII and XIV shows that the temperature coefficient of the production of formaldehyde from substances of the third group is less than unity above 30°, whilst with those of the first group it is more than unity. Moreover, the temperature coefficient of the photosynthesis of formaldehyde from potassium bicarbonate solutions is also comparable with the values obtained with substances of the third group. This is possibly due to the fact that above 30°, the loss of formaldehyde due to decomposition and evaporation becomes more potent than its photosynthesis with the net result that the yield of formaldehyde becomes less and less as the temperature increases. It should

be noted, however, that the percentage of the amount of the substance oxidised goes on increasing with temperature though the yield of formaldehyde follows the reverse order above 30°.

It will be interesting to note in this connection that the values for the temperature coefficient of photosynthesis as going on in the plants is less than unity above 30°. When substances like glycine, critic acid, etc. are oxidised at high temperatures, the yield of formaldehyde goes on increasing though not directly proportional to it, since it happens to be a product of their direct photo-oxidation. Thus from the study ofth e temperature coefficient also, it is concluded that the generation of formaldehyde from substances belonging to the third group is a photosynthetic process.

(*iv*) *The greater the energy generated in the photo-oxidation of a certain organic substance, the greater is the amount of formaldehyde detected during photo-oxidation.* If the view advanced here is correct, then the amount of formaldehyde photosynthetically formed during the photo-oxidation of a certain substance should depend upon the amount of energy evolved during oxidation, since this energy supplies a part of the energy needed for the photosynthesis of the former. This is evident from the results summarised in Table XVI. The amount of formaldehyde, when equivalent solutions of different substances are exposed to air and light, is greatest with salts of fatty acids, then follow the amino-acids and carbohydrates. However, with potassium bicarbonate it is much less. It is well known that the amount of heat generated per gramme of fat oxidised is 9 calories, whilst with both carbohydrates and proteins it is only 4·1 calories per gramme. It is clear, therefore, that the amount of formaldehyde formed during these photo-oxidations increases with the amount of the energy generated in the oxidation of organic substances. That is why the salts of the fatty acids, which are producers of large amount of energy, generate the maximum amount of formaldehyde, while potassium bicarbonate solutions give rise to amounts of formaldehyde which are comparable with the small amount of energy evolved during their decomposition. This is certainly due to the fact that the oxidation of organic matter in ari and light sets free energy, and this energy of oxidation (respiration) is utilised in the photosynthesis of formaldehyde and other products. This experimental observation, therefore, is in favour of the view advanced here.

Why Photosnythesis takes place in Red Light when it requires a Radiation of 2552Å.

It is generally believed that photosynthesis is most active in red and green lights. Wurmser (*Compt. rend.*, 1920, **171**, 920) observed that

photosynthesis was most active in the red region, since the red rays were absorbed by chlorophyll to a greater extent than the blue or violet. These conclusions were corroborated by the researches of Warburg and Negelein (*Z. physikal. Chem.*, 1923, **106**, 191) on chlorella. Thermodynamically the reaction involved in carbon assimilation requires a wave-length of 2552Å, but from the observations of Wurmser and Warburg it is clear that photosynthesis is most active in red light.

It is well known that even in the presence of light, respiration goes on along with photosynthesis in plants. It is probable that a part of the energy evolved during respiration already supplies a part of the energy needed for photosynthesis, thus rendering carbon assimilation possible by longer wave-length as reported by Wurmser and others. Hence apart from its activation by adsorption on chlorophyll surface, carbon dioxide used in photosynthesis may be partially activated by the energy of respiration.

These observations explain the difficulty of obtaining formaldehyde or carbohydrates from carbon dioxide *in vitro*, because the presence of an exothermal reaction, such as, the photo-oxidation of carbohydrates or other organic compounds seems to facilitate this reaction.

Why is Oxygen necessary in Photosynthesis ?

It is generally believed that photosynthesis going on in the plants stops in the complete absence of oxygen. Thus Boussingault ("Agronomie Chimie Agricole et physiologie", Paris, 1868, p. 329), Pringsheim (*Sitz. preuss. Akad. Wiss.*, 1887, p. 763), Willstatter and Stoll ("Untersuchungen uber die Assimilation", Berlin, 1918. p. 349), Warburg (*Biochem. Z.*, 1919, **100**, 230) and Spoehr and McGee (*Carnegie Inst. of Washington Publ*, 1923, p. 325) have all shown that the plants lose their power of assimilation when kept in an oxygen-free atmosphere. It is rather striking that a substance which is produced in carbon assimilation should be indispensable to start it.

It has already been stated that photosynthesis in red light is possible only when a part of the energy required is supplied through respiration. In the absence of oxygen, respiration which supplies a part of the energy needed for photosynthesis will stop and hence the latter will also stop. When oxygen is present, respiration starts with the liberation of energy which is partially used up in photosynthesis. Thus oxygen is not directly used in photosynthesis, but influences it indirectly through respiration. Thus may be the probable explanation of the classical researches of Boussingault, Pringsheim, Willstätter and others. It appears, therefore,

that besides light, carbon dioxide, water and chlorophyll, energy from respiration and consequently oxygen are also necessary for photosynthesis.

Relation between Photosynthesis and Respiration.

Regarding the relation between photosynthesis and respiration, Spoehr and McGee (" Photosynthesis", 1926, p. 143) have stated as follows:—

" The light green varieties had a lower rate of respiration than the normal plants though there was no direct parallelism between chlorophyll content and respiration," and again " Respiration is an important internal factor affecting photosynthesis in addition to the chlorophyll content and the number of chloroplasts," and further " The exact manner in which these two processes may be linked is still an open question." Similar observations have been made by Miss Henrici (Inaugural Dissertation, Basel, 1018) and Boysen-Jensen (*Bot Tidskrift*, 1916, **36**, 319) who report that plants which have a high rate of photosynthesis have also a high velocity of respiration. However, the mechanism in which the two fundamental processes are related is not yet clearly understood.

From the viewpoint already discussed it will be evident that the greater the rate of respiration in a plant, the greater is the possibility of a supply of energy from it for photosynthesis. As already stated, photosynthesis appears to depend partly on the amount of energy produced during the photo-oxidation of organic substances, it is expected that the former will be related by respiration which is a constant source of supply of energy. Thus the way in which the two processes are connected seems to be the supply of energy evolved in respiration to be partially utilised in photosynthesis.

It will be interesting to note in this connection that Krebs and Henseleit (*Z. physiol. Chem.*, 1932, **210**, 33) have reported that the formation of urea from ammonia and carbon dioxide in the animal body is closely related with respiration. According to Borsook and Keighley (*Science*, 1933, **77**, 114), the synthesis of urea from ammonia and carbon dioxide by liver slices is associated with an increase of oxygen absorption.

It is well known that atmospheric nitrogen can only be fixed by some bacteria when supplied with energy from carbohydrates on their oxidation, or some other suitable oxidation reaction. It is of interest to note here that Dhar and Mukerji have recently observed the fixation of atmoshperic nitrogen in the soil when the latter is fed with molasses. They are of the opinion that the oxidation of the sugars present in the molasses induces the combination of atmospheric nitrogen and oxygen ; the first reaction being the supplier of energy for the accomplishment of the latter. The amount of

nitrogen fixation in light is much greater than in the dark, because along with the oxidation energy of ($C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 676$ K. Cal) light is also utilised in the endothermal chemical change involved in nitrogen fixation ($N_2 + O_2 + 53{\cdot}2$ K. Cal $= 2NO$).

In experiments *in vitro*, very little formaldehyde should be expected to be formed from carbonic acid or bicarbonate solutions on exposure to sunlight, because in sunlight short-wave ultraviolet rays exist in small amounts. On the other hand, in presence of ferrous and manganous salts we have been able to detect greater amounts of formaldehyde from carbonic acid than in their absence, because their oxidation in presence of air is an exothermal reaction which supplies a part of the energy needed for photo-synthesis. Hence it is easier to obtain formaldehyde or any other energy-rich organic compound from carbonic acid or bicarbonate solutions on exposure to light when a suitable exothermal reaction is made to take place in the same system. In plants, such an exothermal reaction is respiration.

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COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS. PART II. CHROMIUM BIGUANIDINES.

By Priyadaranjan Rây and Nripendra Nath Ghosh.

A study of the preparation and properties of the salts of chromium *tris*-biguanide with complex inorganic acids has been made. The salts of the tri-basic acids have been observed to form sparingly soluble well-defined crystals and those of the dibasic ones to contain an additional foreign anion.

In Part I of this series the preparation and properties of the chromium *tris*-biguanide base and its salts with simpler acids have been described (Rây and Saha, *J. Indian Chem. Soc.*, 1937, **14**, 670). The constitution of the metal-biguanide complexes has also been discussed in detail in that paper. A study of the salts of chromium *tris*-biguanide with complex inorganic acids has now been made and forms the subject matter of the present communication. Among the salts of the various complex acids studied, it has been observed that those of the tribasic ones form sparingly soluble well-defined crystals; the salts of the di- and monobasic complex acids contain an additional foreign anion. No definite compounds with tetra-basic complex acids could be obtained

The complex salts studied are the ferricyanide, cobalticyanide, chromithiocyanate, cobaltinitrite, chloro-nitroprusside, hydroxo-mercuri-iodide and chloro-bismuthi-iodide. Their composition support the triacidic character of the chromium *tris*-biguanide base. The complex also forms aurichloride and sparingly soluble platinichloride resembling those of organic amines.

Experimental.

Chromium tris-*Biguanide Ferricyanide*.—Concentrated solutions of the complex hydrochloride and potassium ferricyanide were mixed together with constant stirring. After a few seconds dark-brown shining needles commenced to separate These were collected, washed with cold water and dried in *vacuo* over $CaCl_2$. The substance is very sparingly soluble in water. {Found: N, 51·48; Cr, 9·16; Fe, 9·66. $[X]Fe(CN)_6$ requires N, 51·85; Cr, 9·17; Fe, 9·87 per cent}.

Where $X = Cr(BigH^+)_3$, and BigH = one biguanide molecule.

Chromium tris-*biguanide cobalticyanide* was obtained by mixing together concentrated solutions of the complex hydrochloride and potassium cobalticyanide. The rose-coloured crystalline precipitate was

washed first with water and then with alcohol. It was dried as in the previous case. The substance is very sparingly soluble in water. {Found: N, 51·76; Co, 10·26; Cr, 9·33. $[X]Co(CN)_6$ requires N, 51·58; Co, 10·35; Cr, 9·12 per cent}.

Chromium tris-*biguanide chromithiocyanate* was obtained as a light violet crystalline precipitate when strong solutions of the complex hydrochloride and potassium chromithiocyanate were mixed together. It is almost insoluble in water, but highly soluble in acetone and sparingly in alcohol. {Found: N, 36·28; S, 23·71; Cr, 13·08. $[X]Cr(SCN)_6$ requires N, 36·34; S, 23·73; Cr, 12·85 per cent}.

Chromium tris-*biguanide cobaltinitrite* was prepared from the complex hydrochloride and sodium cobaltinitrite as described in the previous cases. It forms very sparingly soluble brown crystals. {Found: Co, 8·66; Cr, 7·38. $[X]Co(NO_2)_6$ requires Co, 8·55; Cr, 7·53 per cent}.

Chromium tris-*Biguanide Chloro-nitroprusside.*—A strong solution of sodium nitroprusside was slowly added to a concentrated solution of the complex hydrochloride. The mixture, on cooling in ice, deposited pink-coloured crystals. The substance is fairly soluble in water and contains also chlorine as an anion. [Found: N, 48·73; Cr, 8·40; Fe, 9·06. $[X]Cl\,[Fe(NO)(CN)_5]$ requires N, 48·47; Cr, 8·59; Fe, 9·23 per cent}.

Chromium tris-*Biguanide Hydroxo-mercuri-iodide.*—A concentrated solution of the chromium *tris*-biguanide hydroxide was treated with a solution of mercuric chloride in potassium iodide (just sufficient to redissolve the mercuric iodide first precipitated). A brick-red crystalline precipitate readily separated from the solution. This was washed and dried as before. The substance is almost insoluble in water, but readily dissolves in alcohol. If the complex hydrochloride be used in place of the base for the preparation of this salt, a substance containing chlorine in the anion is formed. The salt melts at 251° (decomp.). {Found: N, 13·56; I, 48·93, Cr, 3·52; Hg, 26·45. $\left[X\right]^{OH}_{(HgI_3)_2}$ requires N, 13·68; I, 49·67; Cr, 3·38; Hg, 26·07 per cent}.

Chromium tris-*biguanide chloro-bismuthi-iodide* was obtained as an orange-red precipitate by adding slowly a strong solution of the complex hydrochloride to an acid solution of bismuth trichloride in an excess of potassium iodide. This was washed and dried as before. The substance is very sparingly soluble in water and contains also chlorine as an anion. {Found: N, 11·29; I, 56·06; Bi, 22·61; Cr, 2·88. $\left[X\right]^{Cl}_{(BiI_4)_2}$ requires N, 11·50; I, 55·68; Bi, 22·91; Cr, 2·85 per cent}.

An orange-coloured silky crystalline precipitate of aurichloride also was obtained by adding a strong solution of aurichloric acid or sodium aurichloride to a concentrated solution of the complex hydrochloride. This is moderately soluble in water.

Similarly an orange-yellow crystalline precipitate, sparingly soluble in water but readily soluble in dilute acids, was obtained from the complex hydrochloride and platinichloric acid or sodium platinichloride.

With potassium ferrocyanide and thiosulphato-pentacyano-potassium cobaltiate the solution of the complex hydrochloride gives granular precipitates of indefinite composition.

Method of Analysis.

The ferricyanide salt was decomposed by heating with concentrated H_2SO_4. In one part of the prepared solution, iron was estimated volumetrically, and in the other part, the weight of the mixed oxides (Cr_2O_3 and Fe_2O_3) was determined. The nitroprusside compound was also analysed in a similar way.

The cobalticyanide compound was decomposed by heating with concentrated H_2SO_4. Chromium was precipitated from the resulting solution as $Cr(OH)_3$ by pyridine. In the filtrate cobalt was estimated as $CoSO_4$. The cobaltinitrite compound was also analysed in this way.

In the case of chromithiocyanate compound, chromium was precipitated as $Cr(OH)_3$ after decomposing the compound with concentrated H_2SO_4. Sulphur was oxidised to sulphate by ammoniacal H_2O_2 and estimated as $BaSO_4$.

The mercuri-iodide compound was dissolved in HCl. Mercury was precipitated as HgS and estimated as such. In the filtrate chromium was estimated as $Cr(OH)_3$. For iodine, the substance was digested with sodium carbonate solution and in the filtrate iodine was estimated as AgI.

The bismuthi-iodide compound was treated with ammoniacal H_2O_2 to oxidise the chromium ; the latter was then estimated iodometrically in the filtrate. The residue was dissolved in nitric acid and from the solution bismuth was precipitated as basic carbonate. Iodine was estimated as in the case of the mercuri-iodide compound, but the precipitate of silver iodide was digested with dilute ammonia to remove the silver chloride simultaneously precipitated.

Nitrogen was estimated by Kjeldahl's method in all the cases except that of nitroprusside. In the latter compound it was estimated by Dumas' method.

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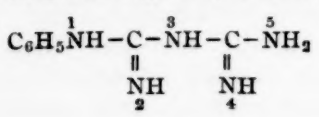
COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS. PART III. CHROMIUM PHENYLBIGUANIDINES.

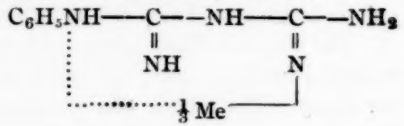
By Priyadaranjan Rây and Nripendra Nath Ghosh.

Preparation, properties and the composition of the chromium *tris*-phenylbiguanide base and its salts have been described in the present paper. The constitution of the complex has also been discussed.

In Part I of this series the preparation, properties and constitution of the chromium *tris*-biguanide base and its salts have been described (Rây and Saha, *J. Indian Chem. Soc.*, 1937, **14**, 670). In the present paper we have studied the properties of the complexes formed by a substituted biguanide, phenylbiguanide (I) with the same metal. The free chromium *tris*-phenylbiguanide base has been prepared and converted into a number of its salts. The properties of these substances differ somewhat from those of the corresponding simple biguanide complexes. The fact that the chromium *tris*-phenylbiguanide forms a stable hydroxide, carbonate and a number of salts which dissociate in aqueous solution like the corresponding ammonium salt, without appreciable hydrolysis, indicates that the co-ordination with the metal atom occurs at (1) and co-valence at (4), as shown in (II). The alternative possibility with co-valence at (2) and co-ordination at (5) would leave only C_6H_5NH-group free to form salts in combination with acids. But such salts would dissociate like aniline salts in solution differing widely from those described in this paper. The basic character of the chromium *tris*-phenylbiguanide complex is, therefore, due to the free amino-group at (5). This, as well as the formation of the anhydrous base—chromium *tris*-phenylbiguanide—furnish additional evidences in support of the constitution of chromium biguanidines or, for the matter of that, of the metal-biguanide complexes in general, as deduced in Part I (Ray and Saha, *loc. cit.*).



(I)



(II)

An interesting fact was observed regarding the properties of the hydrated base. The crude base, which is soluble in alcohol, on recrystallisation from the alcoholic solution becomes insoluble in the same solvent. This seems to suggest a slow polymerisation.

Experimental.

Phenylbiguanide hydrochloride was prepared from aniline hydrochloride and dicyandiamide by heating them in alcoholic solution with a reflux

condenser on a water-bath for about two hours (Smolka and Friedrich, *Monatsh.*, 1888, **9**, 227).

Chromium tris-*Phenylbiguanide Hydroxide.*—Phenylbiguanide hydrochloride (15 g.) was treated with a solution of chromium chloride and a moderately strong solution of caustic soda in excess. The mixture was then heated on the water-bath when a deep red oil commenced to separate; the heating was continued for about half an hour after this. The supernatant alkaline liquid was then poured off and the oily layer was washed several times with ice-cold water in which it was insoluble. The oil gradually solidified to a crystalline mass. It was then extracted with alcohol; the alcoholic filtrate was mixed with a little water and allowed to stand overnight, when the crystals of the base separated in a pure state. The product, thus obtained, did not, however, redissolve in alcohol. The crystals were washed with alcohol and dried over $CaCl_2$ and KOH.

The substance forms rose-red crystals, almost insoluble in water. It reacts alkaline to litmus and liberates ammonia when heated with ammonium salt solutions; m.p. 196° with decomposition. {Found : N, 33·30 ; Cr, 8·19 ; H_2O, 8·67. [X] $(OH)_3$ requires N, 33·12 ; Cr, 8·20 ; H_2O, 8·52 per cent}. where X = Cr $(PhBigH^+)_3$ and PhBigH = one phenylbiguanide molecule.

The substance lost two molecules of water at 110° and became anhydrous at 135°.

Chromium tris-*Phenylbiguanide Hydrochloride.*—The hydrochloride was prepared by just dissolving the preceding base in cold dilute hydrochloric acid avoiding an excess of the latter. The clear solution was cooled in ice, when rose-red crystals of the hydrochloride separated from the latter. These were washed with a little ice-cold water and dried in vacuum over concentrated H_2SO_4. The hydrochloride is soluble in water, but more readily in alcohol and acetone. {Found : N, 30·61 ; Cl, 15·20 ; Cr, 7·37. [X]Cl_3 requires N, 30·45 ; Cl, 15·44 ; Cr, 7·53 per cent}.

Equivalent conductivity at 0°.

V ... 32	64	128	256	512	1024	2048
Λ.... 47·14	52·81	54·12	57·28	59·19	61·45	63·04

Equivalent conductivity of ammonium chloride at 0° amounts to 81·3 mhos at 1024 v and that of 1/3 $La(NO_3)_3$ at infinite dilution at the same temperature is about 67-69 mhos. Hence the result is in good agreement with the adopted constitution for the complex.

Mol. Wt. from the freezing-point.

Substance per 100 g. water	Depression Δ.	Mol. wt. m.	Vant Hoff's factor $i = M/m$.	Degree of dissociation $\alpha = (i-1)/n-1$.
1·015	0·097°	194·8	3·54	0·847
0·508	0·049	192·9	3·57	0·857

From conductivity at corresponding dilutions at 0°, α is approximately 0·81.

Chromium tris-*phenylbiguanide hydrobromide* was prepared from the hydrochloride in cold concentrated solution by the addition of a concentrated solution of KBr. The pale rose crystals were washed and dried as in the previous case. Properties resemble those of the chloride. {Found: Br, 28·94 ; Cr, 6·41. [X] Br_3 requires Br, 29·16 ; Cr, 6·31 per cent}.

Chromium tris-*phenylbiguanide hydriodide* was prepared as in the case of hydrobromide from a cold strong solution of the hydrochloride by the addition of concentrated KI solution. The pale rose-coloured crystals were washed and dried as before. It resembles the other halides in properties. [Found: I, 39·22; Cr, 5·27. [X]I_3 requires I, 39·52; Cr, 5·39 per cent].

Chromium tris-*phenylbiguanide nitrate* was obtained as rose-red crystals from a cold strong solution of the hydrochloride and potassium nitrate. The substance is soluble in water, alcohol and acetone. {Found: Cr, 6·82 ; NO_3, 23·80. [X]$(NO_3)_3$requires Cr, 6·76 ; NO_3, 24·18 per cent}.

Chromium tris-*phenylbiguanide chromate* was prepared like the previous salts from a cold strong solution of the hydrochloride and a concentrated solution of potassium chromate. It separates as biscuit coloured crystals, very sparingly soluble in water and insoluble in alcohol. {Found : Cr(total), 16·80; Cr(as CrO_4), 10·24. $[X]_2(CrO_4)_3$ requires Cr(total), 17·17. Cr(as CrO_4), 10·30 per cent}.

Chromium tris-*Phenylbiguanide Sulphate*.—On treating a solution of the hydrochloride with a solution of ammonium sulphate, the crystalline precipitate, which separated, always contained some chlorine. The pure sulphate was, however, obtained by digesting in the cold the chromium *tris*-phenylbiguanide base with a slight excess ot $N/10$-H_2SO_4 for about 24 hours. The product was washed and dried as usual. It forms pale rose crystals, very sparingly soluble in water. {Found: S, 6·32 ; Cr, 6·93. $[X]_2$ $(SO_4)_3$, $2H_2O$ requires S, 6·44 ; Cr, 7·0 per cent}.

A solution of the chloride was found to give the following precipitate with solutions of

1. Sodium thiosulphate ... ... pale rose-red
2. Sodium bicarbonate or carbonate ... lavender coloured
3. Sodium nitrate ... ... biscuit-coloured
4. Potassium cobalticyanide... ... pale pink
5. Potassium ferrocyanide ... ... biscuit-coloured
6. Potassium ferricyanide ... ... yellow

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COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS PART IV. CHROMIUM *BIS*BIGUANIDINES.

By Priyadaranjan Rây and Haribola Saha.

The present paper deals with the preparation, properties and the constitution of chromium *bis*-biguanidines. The free hydroxo-aquo-chromium *bis*-biguanide has been prepared and found to behave as diacidic base in aqueous solution.

In the previous communications (Rây and Saha, *J. Indian Chem. Soc.*, 1937, **14**, 670; Rây and Ghosh, *this issue*, p.) the preparation, properties and the constitution of chromium *tris*-biguanidines and chromium *tris*-phenylbiguanidines have been reported upon. It was already mentioned (Rây and Saha, *loc. cit*) that the chromium *tris*-biguanide base and its salts undergo, on keeping in dilute aqueous solution, gradual hydrolysis with elimination of a biguanide molecule, giving rise to a new series of *bis*-biguanidine complexes, as represented below :

$$[Cr(BigH^+)_3]X_3 + 2H_2O = [Cr(BigH^+)_2 \cdot OH \cdot H_2O]X_2 + [BigH_2]X,$$

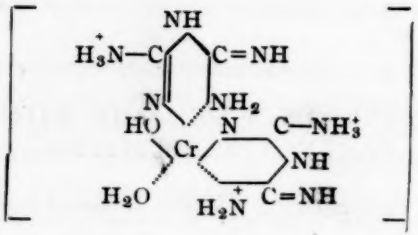
where BigH = one biguanide molecule, X = OH, Cl, Br, etc. This corresponds to the tetrammines of the metalammine complexes, just as the *tris*-biguanidines represent the hexammines. The *bis*-biguanidine complexes form bivalent cations in keeping with the character of *tris*-biguanidines which form tervalent ones. That the formation of complex biguanide bases and salts is due to secondary co-ordination by the free amino-group of the primary metal-biguanide complex is hereby well illustrated.

The chromium *bis*-biguanidines again, in dilute aqueous solution, undergo further hydrolysis on keeping and form complex monobiguanidine products with elimination of a second biguanide molecule. The latter hydrolyse further leading finally to chromium hydroxide.

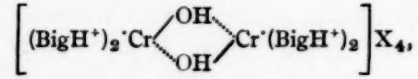
$$[Cr(BigH^+)_2 \cdot OH \cdot H_2O]X_2 + 2H_2O = [Cr(BigH^+) \cdot (OH)_2 \cdot (H_2O)_2]X + [BigH_2]X.$$

It has not yet been possible to prepare this monobiguanide complex in a pure state.

The constitution of the hydroxo-aquo-chromium *bis*-biguanide compounds can be represented by the following figure.



The hydroxo-aquo-chromium *bis*-biguanide hydrochloride, when kept for a long time over concentrated H_2SO_4, or heated to 120°-130°, loses water even from the complex zone, giving rise to a product of the composition $Cr(Big^+H)_2{\cdot}OH{\cdot}X_2$, but no di-ol binuclear compound of the type



as might be expected. For, the anhydro-compound on keeping in air regains rapidly the whole of its original water-content, regenerating the parent hydroxo-aquo-compound. Further-more, the solution of the anhydro-compound is slightly alkaline and liberates ammonia from ammonium salts like the original hydroxo-aquo-compound. A di-olcompound, as is well-known, is neutral and does not liberate ammonia from ammonium salts. This hydroxo-aquo-compound evidently possesses a *cis*-configuration, being produced by the elimination of one bidentate biguanide radicle from the complex zone, which occupied *cis*-positions in the same. This is confirmed by the fact that the complex hydroxo-aquo-compound readily reacts with ethylenediamine giving rise to mixed ammines. That this *cis*-hydroxo-aquo-compound does not give rise to a di-ol complex on dehydration is due to the greater tendency of the Cl-ion from the outer zone to co-ordinate with the central Cr atom than that of the two complex residues to condense with elimination of water.

$$[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O]Cl_2 \longrightarrow [Cl(BigH^+)_2{\cdot}OH{\cdot}Cl]Cl + H_2O.$$

A chlorine ion becomes bound by co-ordination with Cr atom and the complex becomes a univalent cation by this internal compensation. In aqueous solution, however, it rapidly reverts at the ordinary temperature to the original hydroxo aquo type ; freezing-point measurement, on the other hand, indicates its existence at low temperature.

A solution of the hydrochloride, which, as its conductivity shows, dissociates into two ions besides the complex ion, on keeping for a day or

two fails to precipitate silver chloride on addition of silver nitrate. The solution becomes only slightly opalescent; but the whole of the silver chloride is precipitated by the addition of a neutral salt like NH_4NO_3. How can an aged solution of a crystaliine complex salt prevent the formation or growth of silver chloride particles is difficult to account for! The same strange property, as already stated (vide Part I), is also exhibited by the complex *tris*-biguanide hydrochloride and hydrobromide. The *bis*-biguanide hydrobromide, and to a less extent the hydriodide, also behave similarly. Even a fresh solution of the hydrochloride shows a tendency towards such behaviour.

The free hydroxo-aquo-chromium *bis*-biguanide base has been prepared from the sulphate by decomposition with baryta, which, in aqueous solution, behaves as a diacidic base and liberates ammonia from ammonium salts.

Experimental.

Hydroxo-aquo-chromium-bis-*biguanide Sulphate*.—A concentrated solution of chromium *tris*-biguanide hydroxide (7 g.) was treated with a concentrated solution of ammonium sulphate. The sparingly soluble complex *tris*-biguanide sulphate was precipitated with liberation of ammonia. On warming on the water-bath, the latter went into solution in ammonia and, at the same time, gradually hydrolysed into the very sparingly soluble hydroxo-aquo-chromium *bis*-biguanide sulphate, which separated as a violet-coloured crystalline precipitate. This was washed first with hot water and then with alcohol. The product was dried in air. Yield 4 g.

On treatment with warm NaOH solution the substance is converted into chromium *tris*-biguanide hydroxide which separates as ruby-red crystals. The sulphate does not lose any water even when heated to 120°. {Found : N, 36·14, 36·2 ; S, 8·16, 8·17 ; Cr, 13·50, 13·70. $[Cr(BigH^+)_2OH{\cdot}H_2O]SO_4$ requires N, 36·35 ; S, 8·30 ; Cr, 13·50 per cent}.

Hydroxo-aquo-chromium bis-*Biguanide Hydrochloride*.—It was prepared by digesting the above-described hydroxo-aquo-sulphate in slight excess with the solution of a calculated quantity of barium chloride. The mixture was gently warmed on the water-bath and filtered. The filtrate was treated with a few c.c. of a concentrated solution of ammonium chloride and a little alcohol, and then allowed to stand overnight. The rose-red crystals that separated were filtered, washed at first with ice-cold water and then with alcohol. They were afterwards dried in air. The mother-liquor, on keeping, gave a second crop of the hydrochloride.

The same compound was also formed when a concentrated solution of the chromium *tris*-biguanide base was heated on the water-bath with ammonium chloride. The solution, on keeping, deposited crystals of the hydrochloride.

The substance is readily soluble in water. The solution is slightly alkaline to litmus and liberates ammonia from ammonium salts. On treatment with warm caustic alkali solution, the chromium *tris*-biguanide base is formed. Acids lead to decomposition and formation of chromic salts. Freshly prepared concentrated solution gave an immediate precipitate with a solution of silver or thallous nitrate. But from an aged solution (after 24 hours) silver nitrate failed to precipitate AgCl, and gave only a slight turbidity. Addition of an electrolyte like NH_4NO_3 produced, however, an immediate and complete precipitation. On keeping the aged solution mixed with silver nitrate, the colour became darker and a grey precipitate of a mixture of metallic silver and AgCl separated on boiling with ammonium nitrate solution. Metallic silver probably resulted from the reduction of a little Ag_2O, formed by the alkaline reaction of the hydroxo-salt. {Found : N, 36·67, 36·9 ; Cl, 18·70, 18·74 ; Cr, 13·90, 13·85. $[Cr(BigH^+)_2\,OH\cdot H_2O]Cl_2\cdot H_2O$ requires N, 36·50 ; Cl, 18·78 ; Cr, 13·76 per cent}.

An anhydrous variety $[Cr(BigH^+)_2\cdot OH\cdot H_2O]Cl_2$ was obtained in winter season. Keeping over concentrated H_2SO_4 till the weight became constant, the hydrated variety was found to lose 10·2% by weight, which corresponds to a loss of two molecules of water. {Found : N, 41·20 ; Cl, 20·65. $[Cr(BigH^+)_2\cdot OH\cdot Cl]Cl$ requires N, 41·10 ; Cl, 20·76 per cent}.

This anhydrous compound readily regained its original weight on keeping in air. Determination of the molecular weight by the freezing-point method indicates the existence of this chloro-hydroxo-salt in aqueous solution at a low temperature (*vide infra*). With rise of temperature, however, it changes into the hydroxo-aquo compound regenerating the complex bivalent cation as the conductivity values show.

Equivalent conductivity at 28·3°.

v ...	32	64	128	256	512	1024	2048
λ_v ...	95·21	103·7	113·7	122·6	129·7	136·1	141·9

The acido-pentammine chromic salts, which also dissociate into two ions, besides the complex ion, show an equivalent conductivity of 140 mhos at 1000 v at 25°., λ_v for 1/2 $BaCl_2$ and λ_v for NH_4Cl at 1024 v at 25° are also 135 and 147 respectively. The result, therefore, supports the constitution suggested for the complex.

Mol. wt. from the freezing-point.

Substance (anhydrous).	Depression Δ.	Mol. wt (found). m.	Vant Hoff's factor $i = M/m$.	Degree of dissociation $\alpha = (i-1)/n-1$.
0·32 g	0·03°	192	1·875	0·44

The dissociation is rather abnormally weak at 0°. This seems to suggest that at low temperature the hydroxo-aquo salt changes into the chloro-hydroxo variety representing the solid anhydrous compound and dissociates into two ions. In that case $\alpha = 0{\cdot}875$.

Hydroxo-aquo-chromium bis-*biguanide bromide* was obtained as a rose-red crystalline precipitate from a solution of the chloride by the addition of a concentrated solution of NH_4Br in the cold, washed and dried as in the previous case. {Found : Br, 35·20 ; Cr, 11·69. $[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O]Br_2$ requires Br, 35·41; Cr, 11·58 per cent}.

Hydroxo-aquo-chromium bis-*biguanide hydriodide* was prepared like the hydrobromide from a solution of the hydrochloride by the addition of NH_4I. It is a rose-coloured crystalline substance soluble in water. {Found: I, 46·0 ; Cr, 9·76. $[Cr(BigH^+)_4{\cdot}OH{\cdot}H_2O]I_2$ requires I, 46·79 ; Cr, 9·57 per cent}.

Hydroxo-aquo-chromium bis-*biguanide nitrate* was prepared from the sulphate by digestion with the solution of a calculated amount of barium nitrate. The mixture was warmed on the water-bath, filtered and the filtrate cooled in ice, after adding a few c.c. of a concentrated solution of ammonium nitrate and a little alcohol. The violet-red crystals were washed and dried as previousiy described. {Found : N, 40·16; Cr, 12·68. $[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O](NO_3)_2$ requires N, 40·68, Cr, 12·60 per cent}.

Hydroxo-aquo-chromium bis-*biguanide thiosulphate* was obtained as violet-red, sparingly soluble crystals by adding a solution of sodium thiosulphate to that of the complex hydrochloride. {Found : S, 15·84 ; Cr, 12·94. $[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O]S_2O_3$ requires S, 15·96 ; Cr, 12·97 per cent}.

Hydroxo-aquo-chromium bis-*biguanide chromate* was obtained as a dark buff, sparingly soluble, crystalline powder from potassium chromate and the complex hydrochloride. {Found : Cr (total), 24·30; Cr (as CrO_4), 11·90. $[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O]CrO_4{\cdot}H_2O$ requires Cr (total), 24·59, Cr (as CrO_4), 12·29 per cent}.

Hydroxo-aquo-chromium bis-*Biguanide Camphorsulphonate*.—It separated from the filtrate of the normal camphorsulphonate (Rây and Saha, *loc. cit.*) as a rose-red crystalline deposit. {Found : N, 18·70 ; S, 8·70; Cr, 7·10. $[Cr(BigH^+)_2{\cdot}OH{\cdot}H_2O](C_{10}H_{15}O{\cdot}SO_3)_2$ requires N, 18·64 ; S, 8·52 ; Cr, 7·05 per cent}.

Hydroxo-aquo-chromium bis-*biguanide hydroxide* was prepared by digesting the sulphate with a solution of the calculated quantity of barium hydroxide. The mixture was gently warmed on the water-bath and filtered. The filtrate on evaporation *in vacuo* over concentrated H_2SO_4 deposited violet crystals, of which the large-sized ones were carefully picked up and washed several times by decantation with ice-cold water and finally with alcohol. The crystals were dried over a little solid KOH. {Found : N, 42·70; Cr, 16·22. $[Cr(BigH^+)_2 \cdot OH \cdot H_2O](OH)_2$ requires N, 43·30 ; Cr, 16·10 per cent}.

The substance reacts strongly alkaline to litmus and liberates ammonia from ammonium salts. It did not give any precipitate of Ag_2O with silver nitrate solution, due probably to the formation of complex silver compound of a higher order ; but on warming the solution, metallic silver was precipitated with formation of chromate. It lost three molecules of water with partial decomposition when heated to 105°-110°.

A solution of the chloride gave crystalline precipitates with sodium dithionate (silky rose-red), sodium carbonate (rose-red), potassium ferrocyanide (pale rose) and also with potassium ferri- and cobalticyanides.

Hydrolysis of Hydroxo-aquo-chromium bis-*Biguaidines*.—From the filtrate of the hydroxo-aquo-sulphate an insoluble ash-coloured substance separated on dilution and keeping. This was found to contain biguanide and sulphate besides chromium. An analysis of the product gave a ratio of chromium : biguanide : sulphate as 5 : 2 : 1·5.

Attempt was made to prepare the pure chloride from the insoluble sulphate by digesting the latter with the solution of a calculated quantity of $BaCl_2$. A dingy blue-violet filtrate was obtained which, on evaporation to dryness over concentrated H_2SO_4 gave a glassy mass. This on analysis gave a ratio of chromium : biguanide : chloride as 3 : 2 : 2·25 (approx.). The substance is slightly alkaline to litmus.

These are evidently mixtures of polyhydroxy compounds resulting from the gradual hydrolysis of the hydroxo-aquo-chromium *bis*-biguanidines. The final product of the hydrolysis is obviously chromium hydroxide.

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STUDIES IN INDIGOID DYES. PART III.

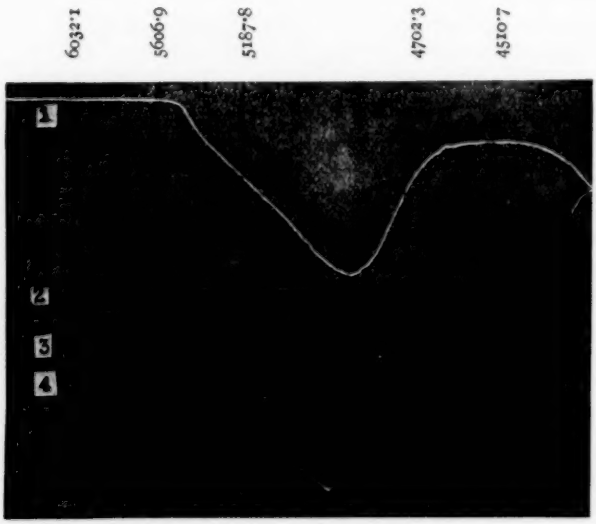
By Sisir Kumar Guha.

The 4-methyl derivatives of *bis*-2-thionaphthene-ethyleneindigo and benzylidene-2-thionaphthene and a few of its substituted products have been prepared. The dyeing shades on cotton and wool obtained from these substances when compared with those from the isomeric 5- and 6-methyl derivatives show that the 4-methyl dyes are lighter in colour than those of the 5-methyl derivatives but deeper than those of the 6-methyl compounds. Quantitative measurement of some of the dyes of the three series confirmed the result. It has also been found that the influence of the methyl group substituted in 4, 5 and 6 positions of the thionaphthene nucleus of the parent compounds is similar to that observed in the acenaphthenequinone series.

The 4-and 6-methyl derivatives of 2-thionaphthene-1′-aceanthryleneindigo have been prepared and their properties studied.

In previous parts of this series (Guha, *J. Indian Chem. Soc.*, 1935, **12**, 659; 1937, **14**, 709) it was found that 5-methyl derivatives of *bis*-

Fig. 1.



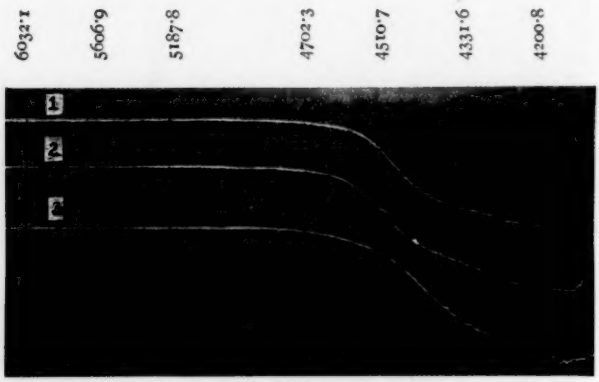
Curves 1-4 refer respectively to *bis*-2-thionaphthene-, *bis*-2(4-methyl)-, *bis*-2(5-methyl)-and *bis*-2(6-methyl)-thionaphthene-ethyleneindigos.

2-thionaphthene-ethyleneindigo and of benzylidene-2-thionaphthene and a few of its substituted products (Friedlander and Risse, *Ber.*, 1914, **47**, 1924; Auwers and Arndt, *Ber.*, 1909, **42**, 543) are deeper in colour than those of the corresponding 6-methyl compounds.

Again in the acenaphthenequinone series (Guha, *J. Indian Chem. Soc.*, 1932, **9**, 423; 1933, **10**, 679; 1936, **13**, 94; 1938, **15**, 20) the influence of one methyl group upon the colour of 2-thionaphthene-acenaphthyleneindigo and its various derivatives, when the methyl substitution occurs in 4, 5 or 6 position of the thionaphthene nucleus, was qualitatively and quantitatively studied. It was observed that the influence of CH_3 substitution in 4 and also in 6 position of the thionaphthene nucleus is to lighten the colour and in 5 position is to deepen the colour of the parent compound.

The present investigation was undertaken to study the effect of CH_3 group in 4-position of the thionaphthene nucleus of *bis*-2-thionaphthene-ethyleneindigo and of benzylidene-2-thionaphthene and its derivatives (*loc. cit.*) with a view to examine if the result is similar to what was observed in the acenaphthenequinone series (Guha, *loc. cit.*). This led the author to condense 4-methyl-3-hydroxythionaphthene (*Chem. Zentrl.*, 928, **99**, I, 759; E. P. 79489; U. S. P. 28037; Guha, *J. Indian Chem. Soc.*, 1938, **15**, 23) with glyoxal, benzaldehyde, *p*-nitro-, and *p*-dimethylaminobenzaldehyde.

FIG. 2.



Curves 1-3 refer respectively to benzylidene-2(4-methyl)-, -2(5 methyl)- and -2(6-methyl)-thionaphthene.

Here also those aldehydes only were used which gave rise to compounds of fairly prominent colours in the 5-and 6-methyl series.

These 4-methyl- derivatives resemble in general the isomeric 5- and 6-methyl compounds in their solubility and in developing shades on cotton and on wool. They also dissolve in concentrated sulphuric acid giving characteristic colours which resemble those developed from the corresponding 5-methyl compounds on similar treatment except the benzylidene-2-(4-methyl)-thionaphthene which develops a deeper colour than those of the corresponding 5- and 6-methyl compounds (Table I). The melting points go on decreasing with the CH_3 groups changing their positions from 4 to 5 and then to 6 position of the thionaphthene nucleus, except benzylidene-2-(methyl)-thionaphthenes where the m. p. rises in the 5-methyl compound and falls down in the 6-methyl derivative. This observation is interesting and quite contrary to that found in the acenaphthenequinone series (*loc. cit.*).

The dyeing shades on wool and on cotton obtained from these 4-methyl derivatives have been found to be deeper than those from the isomeric 6-methyl variety but lighter than those from the corresponding 5-methyl-compounds. Again a comparison of the colours of the dyeings on wool and on cotton of *bis*-2-thionaphthene-ethyleneindigo and of benzylidene-2-thionaphthene and their 4-, 5-, and 6-methyl derivatives on wool indicates that the influence of CH_3 substitution in 4 and 6 position of the thionaphthene nucleus is to lighten the colour and the CH_3 substitution in 5 position deepens the colour. This result is exactly analogous to what was found in the acenaphthenequinone series.

The absorption spectra of some of the compounds of this series have been studied in xylene solution and the absorption curves (Figs. 1 and 2) have been drawn in the same way as mentioned in the acenaphthenequinone series (*loc. cit.*). A study of the curves and the absorption maxima confirmed the earlier qualitative deductions. In the case of the curves in Fig. 2, the position of the change in slope corresponding to the indication of absorption could not be very definitely done as will appear from the trace but the positions were tentatively fixed at the points marked with arrow head.

The absorption maximum of *bis*-2-thionaphthene-ethyleneindigo has been obtained for its various methyl derivatives (Fig. 1).

The thionaphthene-1′-aceanthryleneindigos form with alkaline hydrosulphite a light yellow vat from which light red ochre colour develops on cotton but full shades are not obtained in either of the two.

TABLE I.

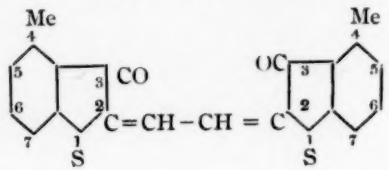
M = *Methyl.* T = *Thionaphthene.* B = *Benzylidene.*

Names.	M. p.	Colour in conc. H_2SO_4.	Dyeing shades on wool.	Dyeing shades on cotton.	Absorption maximum.
Bis-2 T-ethyleneindigo (*cf* Friedlander and Risse, *loc. cit.*)	303°(d.)	Green	Dark red	Dark red	4835A°
Bis-2 (4-M)-T-ethyleneindigo	Above 312°	Deep green	Deep red	Violet red	4810
Bis-2-(5-M)-T-ethyleneindigo	307°(d.)	,,	Dark red	,,	4920
Bis-2-(6 M)-T-ethyleneindigo	300°(d.)	Green	Scarlet red	Deep scarlet red	4765
B-2(4-M)-T	132°	Violet red	Yellow	...	4400
B-2-(5-M)-T	147°	Brownish red	,,	...	4445
B-2-(6-M-)T	134-35°	,,	,,	...	4320
4′-Nitro-B-2(4-M)-T	273°	Greenish blue	Deep yellow	Light orange	...
4′-Nitro-B-2-(5-M)-T	264°	,,	Orange yellow	Do.	...
4′-Nitro-B-2-(6-M)-T	228-29°	Purple	Deep yellow	Yellowish orange	...
4′-Dimethylamino-B-2(4-M)-T	202°	Blue	Cinnabar red	Pink	...
4′-Dimethylamino-B-2(5-M)-T	198-99°	,,	,,	,,	...
4′-Dimethylamino-B-2(6-M)-T	193°	Violet red	,,	Light pink	...

EXPERIMENTAL.

The 4-methyl-indigoid dyes described here were prepared in the same way as the isomeric 5-and 6-methyl derivatives recorded in Parts I and II of this series.

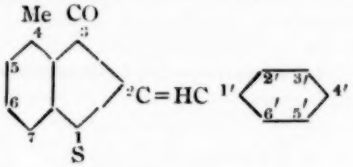
bis-2-(4-Methyl)-thionaphthene-ethyleneindigo.



It separated in fine deep red needles from the reaction mixture consisting of glyoxal sodium bisulphite (0·568 g.) in 15 c.c. of water and 4-methyl-3-hyhroxythionaphthene (0·656 g.) in hot absolute alcohol (35 c.c.) and concen-

trated hydrochloric acid (10 c.c.). It was crystallised from toluene, m.p. above 312°. It is difficultly soluble in chloroform, amyl alcohol and moderately soluble in glacial acetic acid. It dyes wool in deep red shades from an acid bath and cotton in violet red shades from a deep yellow hydrosulphite vat. (Found : S, 18·51. $C_{20}H_{14}O_2S_2$ requires S, 18·28 per cent).

Benzylidene-2-(4-methyl)-thionaphthene.



It was prepared from benzaldehyde (0·424 g.), 4-methyl-3-hydroxythionaphthene (0·656 g.) in hot absolute alcohol (11 c.c.) and concentrated hydrochloric acid (2·5 c.c.), and crystallised from dilute alcohol in fine yellow rectangular crystals, m.p. 132°. It is soluble in petroleum ether. The solution in concentrated sulphuric acid is violet-red. It dyes wool in yellow shades from an acid bath. (Found : S, 13·06. $C_{16}H_{12}OS$ requires S, 12·69 per cent).

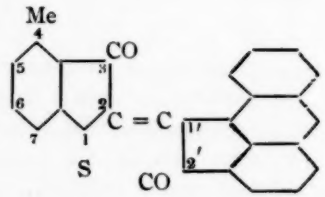
4'-Nitrobenzylidene-2-(4-methyl)-thionaphthene was obtained as yellow-orange small needles from *p*-nitrobenzaldehyde (0·453 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.) in 20 c.c. of hot absolute alcohol and concentrated hydrochloric acid (3 c.c.) and crystallised from glacial acetic acid, m.p. 273°. It is difficultly soluble in benzene and toluene. It dyes wool in deep yellow shades from an acid bath and cotton in light orange shades from a light brown hydrosulphite vat. (Found: S, 10·94. $C_{16}H_{11}O_3NS$ requires S, 10·77 per cent).

4'-Dimethylaminobenzylidene-2-(4-methyl)-thionaphthene separated as its hydrochloride in shining greenish yellow rectangular precipitate from *p*-dimethylaminobenzaldehyde (0·566 g.) and 4-methyl-3-hydroxythionaphthene (0·624 g.) in 13 c.c. of hot absolute alcohol and concentrated hydrochloric acid (1·6 c.c.). It gave on washing with dilute alcohol a bright vermillion dye (0·9372 g.). It crystallised from dilute acetic acid in diamond shaped shining crystals, m. p. 202°. It is difficultly soluble in petroleum ether. It dyes wool in cinnabar-red shade from an acid bath and cotton in pink colour from a faintly red vat. (Found : S, 10·65. $C_{18}H_{17}ONS$ requires S, 10·84 per cent).

bis-2-Thionaphthene-ethyleneindigo was obtained by heating on the water-bath for 1½ hours a mixture of glyoxal sodium bisulphite (1·136 g.) in

30 c.c. of water and 3-hydroxythionaphthene (1·4 g.) in 50 c.c. of hot absolute alcohol and concentrated hydrochloric acid (20 c.c.). The silky dark-red crystalline precipitate (0·731 g.) was crystallised from nitrobenzene in rectangular plates, m.p. 303° (decomp.). It dissolves in concentrated sulphuric acid with a green colour and dyes wool in dark-red shades from an acid bath and cotton in the same shade from a deep-yellow alkaline hydrosulphite vat (*cf.* Friedlander and Risse, *loc. cit.*). (Found : S, 19·63. $C_{18}H_{10}O_2S_2$ requires S, 19·87 per cent).

2-(4-Methyl)-thionaphthene-1'-aceanthryleneindigo.



It separated as dark-brown microscopic needles from the reaction mixture consisting of aceanthraquinone (0·696 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.), 120 c.c. of glacial acetic acid and 5 c.c. of concentrated hydrochloric acid, and was crystallised from nitrobenzene, m.p. above 305°. It is sparingly soluble in acetone, carbon tetrachloride, moderately soluble in amyl alcohol. It dissolves in concentrated sulphuric acid with a green colour and dyes wool in dark-brown shades from an acid bath. (Found : S, 8·12. $C_{25}H_{14}O_2S$ requires S, 8·46 per cent).

2-(6-Methyl)-thionaphthene-1'-aceanthryleneindigo was similarly prepared from aceanthraquinone (0·588 g.) and 6-methyl-3-hydroxythionaphthene (0·416 g.) in 85 c.c. of acetic acid and 5 c.c. of concentrated hydrochloric acid as red-brown microscopic needles and crystallised from xylene. The solution in concentrated sulphuric acid is yellowish-green. It dyes wool in red-brown shades from an acid bath. It resembles in properties the above compound. (Found : S, 8·41. $C_{25}H_{14}O_2S$ requires S, 8·46 per cent).

My thanks are due to Prof. Kamta Prashad for taking interest in this work and to Prof D. K. Bhattacharya and Mr. B. N. Ghosh for helping me with spectrograph and microphotometer.

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STUDIES IN THIXOTROPIC GELATION OF THORIUM MOLYBDATE GELS.

By Mata Prasad, S. M. Mehta and Miss H. Rathnamma.

The viscosity of thorium molybdate gels, which are thixotropic, has been measured during the process of gel-formation using Scarpa's method. The effect of change of concentrations of thorium nitrate, potassium molybdate and hydrochloric acid, of temperature and of the addition of electrolytes and non-electrolytes has also been examined. It is found that the viscosity-time curves are not continuous but show well-marked changes of direction. This is the first instance of its kind observed during the process of gel-formation.

Although Seifriz, Schalek and Freundlich were attracted by the phenomenon of concentrated sols giving rise to gels which liquefy on shaking, Schalek and Szegvari (*Kolloid Z.*, 1923, **32**, 318) were the first to point out the significance of this phenomenon. Several investigators have since attempted to study thixotropic gelation from various points of view. The principal workers on the subject are Freundlich and co-workers (*Kolloid Z.*, 1927, **41**, 102; *Koll.-Chem. Beih.*, 1927, **53**, 231; *Z. physikal. Chem.*, 1932, **160**, 469; *Kolloid Z.*, 1928, **44**, 309; **45**, 348) who investigated the thixotropic behaviour of gels of ferric and aluminium oxides and of bentonite suspensions under different conditions and also examined the effects of the addition of electrolytes and non-electrolytes to them. Hauser (*Kolloid Z.*, 1929, **48**, 57) and Deutsch (*Z. physikal. Chem.*, 1930, **150**, 161) studied the influence of the dimensions of the containing vessel on the thixotropy of gels, the latter stating that this effect is only apparent. Kallmann and Kreidl (*Z. physikal. Chem.*, 1932, **159**, 322) measured the dielectric constant of vanadium pentoxide sol and found that it was diminished by 6% when the gel was formed but Kistler (*J. Phys. Chem.*, 1931, **35**, 815) found no change in the dielectric constant of thixotropic sols before and after gelation. Heymann (*Trans. Faraday Soc.*, 1936, **32**, 462) carried out dilatometric investigations during the sol-gel transformation of ferric hydroxide and found that there was no change in volume. Marinesco (*Compt. rend.*, 1932, **194**, 1824) studied the effects of ultrasonic waves on thixotropic gelation and found that the mechanical disturbances produced are due to interference of the direct and reflected waves.

It is evident that in these sol-gel transformations the total volume of the system remains the same and hence the average distance between the constituent particles does not alter. The development of rigidity in a

fluid sol or the mechanism of thixotropic gelation has been explained by various school of workers in different ways. Satya Prakash and Biswas (*J. Indian Chem. Soc.*, 1931, **8**, 549) who investigated the thixotropic behaviour of the gels of molybdate, phosphate and arsenate of thorium have explained thixotropy on their 'hydration-agglomeration hypothesis' of jellies. According to them the phenomenon of thixotropy cannot be explained on the view put forward by von Weimarn (*Rep. Imp. Ind. Res. Inst. Osaka*, 1921, **9**, 1) that the jellies are formed by the release of highly developed supersaturation.

Ostwald (*Kolloid Z.*, 1928, **46**, 263), Hauser (*J. Rheology*, 1931, **2**, 5), Freundlich ("Kapillar Chemie," 1932, II, 624) and Kistler (*loc. cit.*) explain thixotropic gelation on the consideration that the particles in the gel are surrounded by thick rigid envelopes of orientated water molecules or "lyospheres" which are destroyed by shaking and reformed on allowing the gel to stand; the gel is rigid when the lyospheres are large enough to make contact with each other throughout the system. The only evidence adduced for this theory is the observation by means of an ultra-microscope that during gel formation the Brownian movement of the gelling particles slows down and eventually ceases.

An alternative theory of thixotropic gelation has been put forward by Russell and Rideal (*Proc. Roy. Soc.*, 1936, **A 154**, 540) who have collected evidence in favour of the view that thixotropic gelation is due to orientated coagulation. According to this theory the ultimate particles of which the gel-forming material is composed are anisotropic or anisometric or both and the charge and the adsorbed water molecules, if any, are unequally distributed. Adhesion occurs when two particles approach each other at particular orientation and three dimensional net-work or honey-comb structure, depending upon the nature of the particles, consisting of regularly orientated entities spread throughout the system, is formed. Any stress, such as shaking, destroys the orientation and the structure is reformed when the stress is removed. These authors have examined their theory in respect of all the known facts regarding thixotropic gelation and have found that it offers a satisfactory explanation for all of them.

Thomas Graham first pointed out in 1864 that viscometer could be employed as a colloidoscope. In 1913 Ostwald (*Trans. Faraday Soc.*, 1913, **9**, 34) emphasised the use of viscosity as a property suitable for the study of changes in a colloidal system. In recent years increasing attention has been paid to the study of this property which has been utilised for investigating (*i*) the mechanism of coagulation of sols, (*ii*) the degree of hydration of the colloid particles and (*iii*) the changes which take place during gelation.

CONSTITUENTS OF THE SEEDS OF *BLEPHARIS EDULIS*, PERS. PART II. THE COMPOSITION OF THE OIL. (A CORRECTION).

By G. P. Pendse and Jagraj Behari Lal.

The formula, $C_{27}H_{47}O$, $2H_2O$, was erroneously ascribed to the sterol described in the above communication (*J. Indian Chem. Soc.*, 1937, **13**, 365). The correct formula appears to be $C_{27}H_{42}O_3$. (Found in air-dried samples: C, 78·01, 78·04; H, 10·34, 10·30. $C_{27}H_{42}O_3$ requires C, 78·26; H, 10·15 per cent).

The following data should be inserted at the beginning of page 366.

Table VIII.

Percentage of Saturated Acids in the Oil.

Acid	Found in the saturated acid fraction.	Found in the total fatty acids.	Found in the original oil.
Palmitic	43·44%	5·38%	4·93%
Stearic	54·72	6·77	6·20
Arachidic	0·134	0·017	0·015
Unsaturated	1·544	0·191	0·175

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A study of the literature shows that very scanty data are available on the viscosity measurements of gel-forming mixtures which give rise to thixotropic gels. Miller (*J. Rheology*, 1932, **3**, 163) has investigated the thixotropic properties of paints viscosimetrically and noticed that the maximum fluidity immediately after violent agitation changes too rapidly for measurement.

In this investigation the viscosity of thorium molybdate gel-forming mixtures is measured at different intervals during the process of gel-formation. These gels have been found to be thixotropic by Satya Prakash and Biswas (*loc. cit.*). The effect of change of concentrations of thorium nitrate, potassium molybdate and hydrochloric acid, of temperature and of the addition of electrolytes and non-electrolytes on the viscosity of the gel-forming mixtures has also been examined.

EXPERIMENTAL.

The capillary methods of measuring viscosity have often been criticised because different results are obtained with colloidal systems by using capillary tubes of different diameters. Thus viscosity values of such systems measured by capillary viscometers have lost their usual significance and are now considered to be only empirically indicating the progress of coagulation or gelation. The shape of the viscosity-time curves is again dependent on the number of readings taken in a given interval of time (*cf.* Mardles, *Trans. Faraday Soc.*, 1923, **18**, 327).

Scarpa's method modified by Prasad, Mehta and Desai (*J. Phys. Chem.*, 1932, **36**, 1394) was employed for the determination of the viscosity. The dimensions of the viscometer were as follows :

Volume of the bulb	=1·54 c.c.
Diameter of the capillary	=1·264 mm.
Length of the capillary	=6·42 cm.

The time t_1 required by the mixture to rise through the capillary tube between two fixed marks at constant pressure (14·5 cm. of water) and the time of fall at atmospheric pressure t_2, were measured at different intervals during the process of gel-formation. These intervals (in minutes) were the mean of the time at which the mixture was made to rise and that at which it reached the lower mark while falling since mixing the gel-forming constituents. The viscosity η was calculated from the relation

$$\eta = K\frac{t_1 t_2}{t_1 + t_2}$$

in which the constant K was experimentally determined by using a 60% solution of sucrose, the viscosity of which at 35° is given as 0·2662 (International Critical Tables, **V**, p. 23).

All chemicals used were of Kahlbaum's or Merck's extra pure quality ; potassium molybdate was obtained from the British Drug Houses. These chemicals were used as supplied by the manufacturers without further purification.

10% Solution of potassium molybdate, 2N-HCl and thorium nitrate solution (12 g. in 250 c.c., thoria content 2·24%) were made in redistilled water and were used throughout the investigation.

Preparation of Gels.

5 C.c. of the solution of thorium nitrate were taken in one test-tube and in another were taken solutions of potassium molybdate and hydrochloric acid together with the requisite quantity of distilled water so that after mixing the contents of the two test-tubes the total volume of the mixture was 10 c.c. The two test-tubes, with their contents were first placed in a thermostat maintained at 35° until they attained the constant temperature. They were then mixed by transferring the contents of the second test-tube into the first and back again three times. This manner of mixing was followed every time throughout the investigation. The mixture was then introduced into the viscometer bottle and the readings were immediately taken.

The results obtained are shown graphically :

Fig. 1. Effect of the addition of different amounts of potassium molybdate to the mixture containing 0·24 g. of thorium nitrate and 0·04N-HCl.

Fig. 2. Effect of the addition of different amounts of thorium nitrate to the mixture containing 0·05 g. of potassium molybdate and 0·04N-HCl.

Fig. 3. Effect of the addition of HCl of different concentrations to the mixture containing 0·24 g. of thorium nitrate and 0·05 g. of potassium molybdate.

These curves show that the viscosity of thorium molybdate gel-forming mixtures increases rapidly at first, then decreases and again increases and so on. Thus the variation in viscosity of these mixtures with time is not a continuous function but there are well marked changes in direction.

From measurements of refractivity Joshi and co-workers* have shown for the first time that the progress of slow coagulation of sols and emulsions is not time-continuous but zonal. They regard slow coagulation to be more complex than a simple continued micellar coalescence contemplated by Smoluchowski and ascribe the zonal effect, in part, to building and unbuilding of the micelles during coagulation.

This is the first instance of its kind observed during the process of the formation of gels. Considering the fact that thorium molybdate gel is thixotropic, it will yield to any slight mechanical treatment, such as the one involved in sucking the mixture through the capillary tube. There will be a breaking up of the structure of the gel occasionally and this will cause the observed discontinuities in the viscosity-time curves. It appears that the discontinuous viscosity-time curve is a characteristic of highly thixotropic gels. This viewpoint is confirmed by the viscosity measurements of ceric phosphate gel-forming mixtures. These gels were prepared by Desai (unpublished work) for the first time by mixing solutions of ceric nitrate and phosphoric acid and were found to be thixotropic. The results are shown graphically in Fig. 4. If the gel is not highly thixotropic, *i.e.* it requires large force for its structure to break up, the viscosity-time curve may be a continuous one as has actually been observed in the case of the thixotropic thorium phosphate gel (*cf.* Mehta, Prasad and Parmar, *J. Indian Chem. Soc.*, 1936, **13**, 128).

One remarkable feature of all the curves is that the initial increase in viscosity is continuous in all cases. The time corresponding to the first maximum viscosity is compared with the thixotropic time of set in the following table from which it will be seen that the thixotropic action comes into play only when the specific structures are beginning to form.

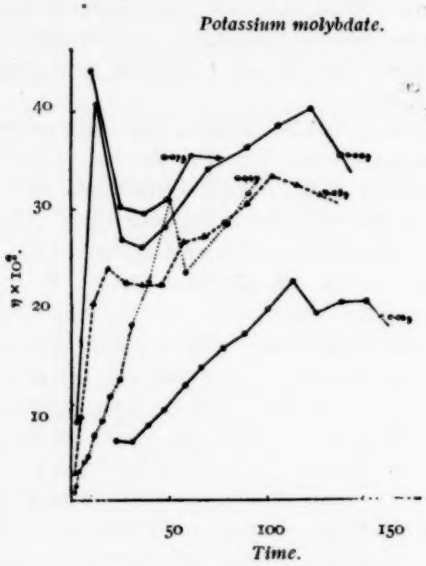
TABLE I.

Thorium nitrate = 0·24 g. HCl = 0·04*N*.

Potassium molybdate (in 10 c c. of the mixtures)	0·03g.	0·04g.	0·05g.	0·06g.	0·07g.
Time of set	185′	100′	65′	45′	32′
Thixotropic time of set	80′	50′	15′	7′	5′
Time corresp. to 1st maximum	113′	48′	18′	8′	—

* *J. Indian Chem. Soc.*, 1936, **13**, 141, 309, 775; 1937, **14**, 103; *J. Bombay Univ.*, 1935, **4**, 140; *Kolloid Z.*, 1936, **75**, 145; *Fettchem. Umsch.*, 1936, **3**, 36. "viscosity" *J. IndiaF Chem Soc.*, 1933, **10**, 329, 599, 699; 1934, **11**, 133, 555, 573, 797; *J. chim. phys.*, 1935, **32**, 455; *Proc. Acad. Sci. U. P.*, 1935, **5**, 41). "Opacity" (*J. Indian Chem. Soc.*, 1936, **13**, 311; 1937, **14**, 388; *Current. Sci.*, 1936, **4**, 870.

FIG. 1.



It will be noticed in Fig. 1 that the viscosity corresponding to the first maximum is greater with mixtures containing larger quantities of potassium molybdate. This is due to the large number of micelles present in the gel-forming mixtures on account of the formation of greater quantity of thorium molybdate. Similar observations are made in curves in Fig. 2 and this may also be caused by an increase in the number of micelles. However, this behaviour of thorium ions in thorium molybdate gels is different from that in gels of thorium phosphate in which case these ions act as peptising agents and as such increase the degree of dispersity and the density of charge on the micelles and lower the rate of increase of viscosity. The difference in the two cases lies in the nature of the coagulating electrolyte ; in the former it is potassium nitrate which gives a high concentration of nitrate ions and in the latter it is nitric acid which is comparatively less ionised. The abundance of the coagulating ions in thorium molybdate gels overcomes the peptising action of thorium ions.

FIG 2.

Thorium nitrate.

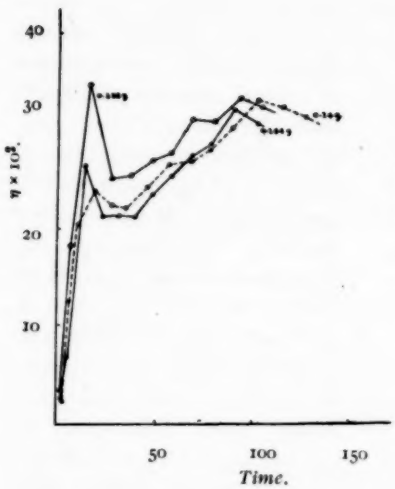
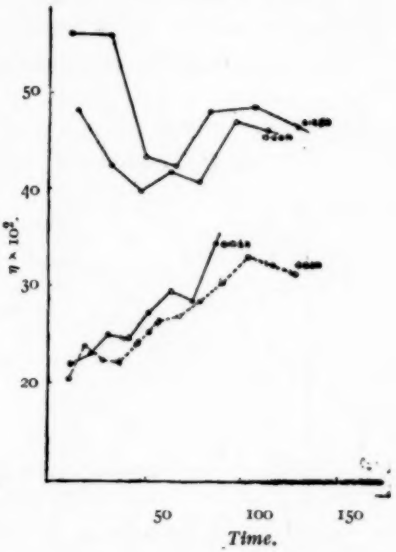


FIG. 3.

Hydrochloric acid.



Hydrogen ions (Fig. 3) tend to increase the value of the first maximum abruptly as the concentration of HCl in the mixture is increased to 0·05*N* but this drops down when it is further increased to 0·06*N*. An increase in the rate of change of viscosity with increase in the concentration of the acid has also been observed in the case of thorium phosphate gels and is caused by the coagulating action of H^+ ions. Greater concentration of H^+-ions act as peptising agents and cause a lowering in viscosity.

Effect of Electrolytes, Non-electrolytes and their Mixtures.

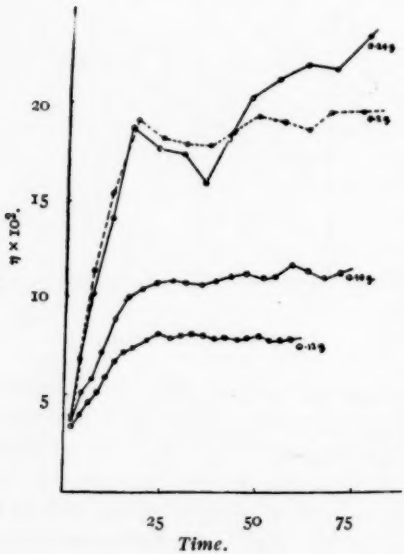
Electrolytes, non-electrolytes and mixtures of these are known to alter the viscosity of a colloidal system in the most varied manner (*vide*, Ostwald, *Trans. Faraday. Soc.*, 1913, **9**, 34), Dhar (*J. Indian Chem. Soc.*, 1928, **6**, 641) found that the viscosity of a number of metallic hydroxide sols decreases on the addition of peptising electrolytes. Dhar and Chakravarty (*Kolloid Z.*, 1928, **44**, 225) found an irregular change in viscosity of silicic acid sols with increasing quantities of electrolytes. Prasad, Mehta and Desai (*J. Phys. Chem.*, 1932, **36**, 1384) measured the viscosity of silicic acid gel-forming mixtures and found that there is an increase or decrease in the rate of change of viscosity on increasing the concentration of acetic

acid in alkaline or acidic mixtures. Mehta, Parmar and Prasad (*loc. cit.*) have studied the effect of the addition of acids and sodium chloride on the viscosity of thorium phosphate gel-forming mixtures and have found that they increase the rate of change of viscosity with time.

The effect of addition of non-electrolytes and of mixtures of non-electrolytes and electrolytes appears to have received special attention of colloid chemists. Billitzer's work (*Z. physikal. Chem.*, 1903, **45**, 312) on a negatively charged platinum sol has shown that it can be discharged and charged positively by varying the amounts of alcohol. Lævites (*Z. Chem. Ind. Kolloide*, 1908, **2**, 237) found that non-electrolytes retard the sol-gel transformation of gelatine and agar-agar. Prasad and Hattiangadi (*J. Indian Chem. Soc.*, 1929, **6**, 991) observed the accelerating effect of alcohols on the alkaline mixtures and a retarding effect on acidic ones. Mehta, Parmar and Prasad (*loc. cit.*) found that the rate of change of viscosity of thorium phosphate gel-forming mixtures decreases as increasing amounts of the non-electrolytes are added to them.

FIG. 4.

Cerium phosphate.



In the present investigation the required quantities of the electrolytes, non-electrolytes and their mixtures were added to the mixture containing 0·24g. of thorium nitrate, 0·05g. of potassium molybdate and 0·04*N*-HCl. The results are shown graphically as follows :

Fig. 5. Effect of different volumes of 0·01*N*-KCl and K_2SO_4.

Fig. 6. Effect of methyl, ethyl and propyl alcohols, glycerine, and 10 % solution of sucrose.

Fig. 7. Effect of methyl, ethyl and propyl alcohols, in the presence of KCl and K_2SO_4.

It will be observed from these figures that the addition of electrolytes, non-electrolytes and their mixtures does not essentially alter the nature of the viscosity-time curves. A few observations made in each case are recorded below.

FIG. 5.

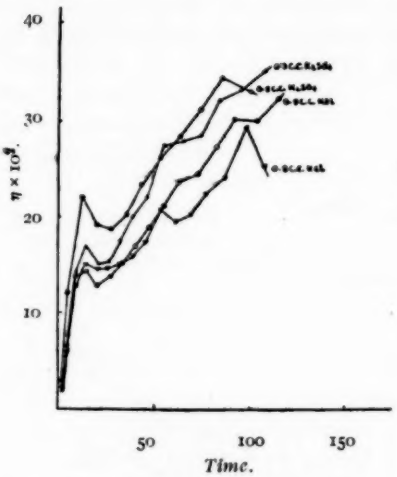
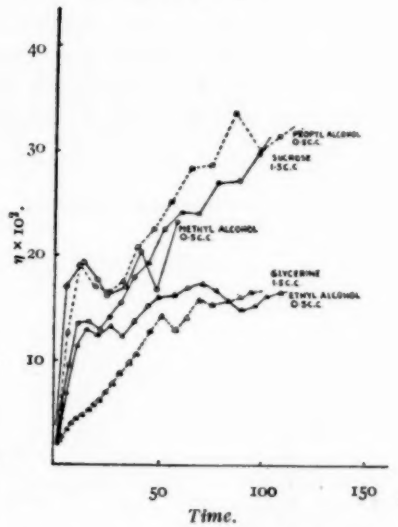


FIG. 6.

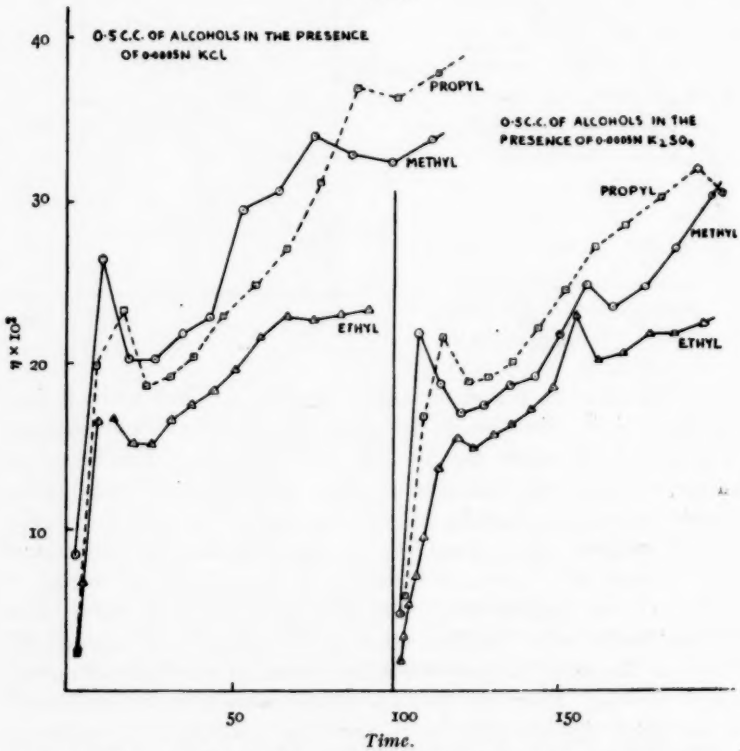


It will be noted in Fig. 5 that after the first fall in viscosity it rises continuously almost linearly in most cases, and the ultimate value reached is, in all cases, much greater than the first maximum value. This behaviour is different from that observed when no extra electrolytes are added to the gel forming mixtures The added electrolytes hasten the rate of coagulation (this is supported by a comparison of the relative effect of the same amount of KCl and K_2SO_4 and thereby increase the rate of change of viscosity. It may happen that stiffer structures are formed under these conditions so that after the first break in the viscosity-time curves, the stiffness of the structure overcomes the stress involved in taking the viscosity reading. From the closeness of the various curves (not shown) it is concluded that the addition of increasing quantities of electrolytes does not magnify the nature of the effect to a large extent.

The effect of the addition of non-electrolytes shown in Fig. 6 is similar to that of the electrolytes, as in these cases also the viscosity increases to a value much greater than that corresponding to the first maximum. No remarkable change has been observed in the vicosity-time curves when

increasing quantities of the non-electrolytes are added to the gel-forming mixtures (curves not shown) : the various curves are quite close to each other except with glycerine in which case they lie fairly apart. The only important observation that has been made is that while the viscosity value at the first maximum decreases as the quantity of ethyl and propyl alcohols and that of glycerine in the mixture is increased, it increases with the addition of methyl alcohol and sucrose solution. The peculiarity of alcohols is not surprising as they have been found to behave specifically under different circumstances but the behaviour of sucrose, which is a well known peptising agent, is certainly striking.

FIG. 7.



The effect of the addition of both electrolytes and non-electrolytes to the gel-forming mixtures is shown in Fig. 7. The presence of electrolytes hastens the coagulation of colloidal solutions in the gel-forming mixture and when non-electrolytes, some of which also behave in the same way, are added to the mixture, the rate of coagulation becomes very rapid

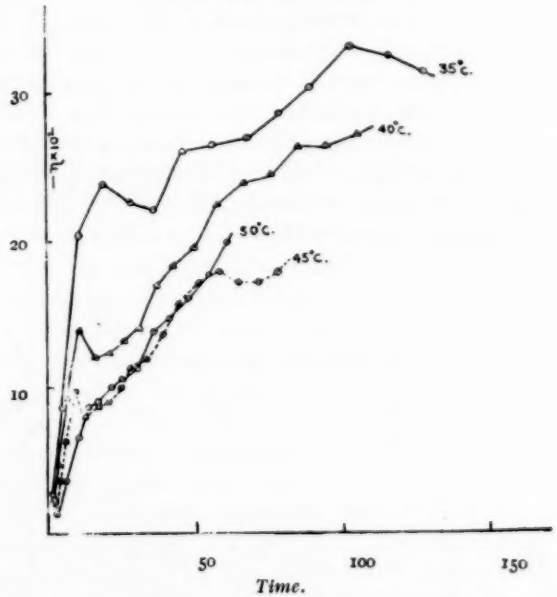
and stiffer gels may be formed. This is not clear from the curves (Fig. 7) which definitely show maxima.

Effect of Temperature.

The solubility of the disperse phase, the hydration of colloidal particles and the rate of coagulation of colloidal solutions undergo a change with a variation in the temperature at which they are measured. The process of gel-formation as suggested by various workers and by Prasad and Hattiangadi (*J. Indian Chem. Soc.*, 1929, **5**, 893, 991) consists of several stages and two of them are the formation of colloidal particles and an increase in their hydration. It will, therefore, be found that the entire course of gelation will be modified by a change in the temperature at which it is studied.

FIG. 8.

Viscosity of the gel-forming mixtures at different temperatures.
(9·05 g. Pot. molybdate).



Satya Prakash (*J. Indian. Chem. Soc.*, 1932, **9**, 193) found that in general the time of setting of inorganic jellies decreases as the temperature is raised with the exception of the jellies of thorium arsenate, vanadium pentoxide and mercuric sulphosalicylic acid which take a longer time to set at higher temperatures. Fleming (*Z. physikal. Chem.*, 1902, **41**, 427)

and Holmes (*J. Phys. Chem.*, 1918, **22**, 516) found that the time of set of silicic acid gel forming mixture is increased with a slight increase in temperature, while Fells and Firth (*J. Phys. Chem.*, 1925, **29**, 243) reported that over the range of temperatures from 0° to 45°, the setting of silicic acid gels does not show a distinct variation with temperature. Szegvari and Schalek (*Kolloid Z.*, 1923, **32**, 318; **33**, 326) also observed that the time of setting of an iron oxide gel decreases with rise of temperature.

The viscosity measurements of dilute sol of albumin at different temperatures by Ostwald (*Trans. Faraday Soc.*, 1913, **9**, 34) showed that with rise of temperature it decreases at first in a normal manner, then increases and again decreases and the increase in viscosity takes place shortly before the appearance of turbidity. Ostwald has also given data on the viscosity measurements of a suspension of starch during heating wherein there is a normal decrease in viscosity in the beginning and then a sharp break followed by a regular increase.

The results obtained with thorium molybdate gels are shown graphically in Fig. 8 for mixtures containing 0·24 g. of thorium nitrate, 0·05 g. of potassium molybdate and 0·04*N*-HCl. It will be seen that the curves beyond the first maximum tend to smoothen as the temperature is raised. Also the value of the first maximum gets less with rise in temperature. This is in accordance with the view that the rise in temperature decreases the hydration of the gelling particles. The consequent decrease in the time of setting of these gels, determined by Fleming's method, with rise in temperature is shown in the following table .

TABLE II.

Thorium nitrate = 0·24g HCl = 0·04*N*.

Pot., molybdate →	0·03g.	0·04 g.	0·05 g.	0·06 g.	0.07 g.
Temp.	Time of set				
35°	162′	87′	62′30″	42′0″	28′0″
40°	150′	85′	61′45″	39′30″	23′30″
45°	135′	65′	46′0″	30′0″	15′0″
50°	120′	50′	34′15″	9′30″	6′15″

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ELECTRODEPOSITION OF LEAD ON BASE METALS. PART I. BEHAVIOUR OF ALKALINE BATHS WITH IRON CATHODES AT LOW CURRENT DENSITIES.

By Shridhar Sarvottam Joshi and T. V. Subba Rao.

A method for the electrodeposition of lead by using moderately concentrated solutions of litharge in aqueous alkali, the effects of current density, temperature, ad lition agents and intermediate coating of metallic antimony have been studied.

The wide and increasing demand for lead coated wares in war and especially in industrial practice has stimulated considerable work for obtaining smooth and coherent deposits of lead. The methods at present in vogue are (*i*) the hot dipping process, (*ii*) spraying, and (*iii*) electrodeposition. Despite the apparent simplicity and directness of the technique involved in (*i*) and (*ii*), they suffer from the disadvantage that the coating is not always uniform and the deposit not firm and adherent all over. This leads to small cracks and air inclusions especially in (*ii*) which necessitates the use of a blast of air or of some other gas, which exposes the protected material to outside action. This factor is obviously of very great importance in the case of lead coated wares which are used for stocking acids and similar reagents of common occurrence in numerous chemical industries, *e.g.*, in lead fittings of storage batteries. A limitation also obtains in (*ii*) since deposition by spraying depends to an appreciable extent on the specific nature of the material to be coated. These difficulties are comparatively absent in (*iii*), since the rate of lead deposition can be made as small as possible ; receptacles with engraving or indents are coated more easily by (*iii*) than by (*i*) and (*ii*). Among the methods for the electrodeposition of lead, the use of acid baths using solutions of a nitrate, acetate, lactate, perchlorate, fluosilicate or a fluoborate is notable. Comparatively satisfactory results have been obtained, however, with the last three substances. An important circumstance observed in this connection is that the concentration of the acid determines very appreciably the nature of the deposit as also the influence of secondary reactions. Thus for example, Smith (*J. Amer. Chem. Soc.*, 1905, **27**, 1287) found that lead which is deposited first as a metal on the cathode is gradually dissolved on prolonged electrolysis and is deposited as the peroxide on the anode. Schuchet (*Z. anal. Chem.*, 1883, **22**, 488) found that lead is precipitated from strongly acid solutions principally as peroxide on the anode, whereas

from feebly acid solutions, the deposits contain, besides the peroxide, an appreciable proportion of the metal. A method based on this observation has been developed recently in these Laboratories for the quantitative estimation of lead from the anodic deposit of peroxide which will be published shortly. These results have been confirmed by the data obtained in the case of the fluoborate, fluosilicate and perchlorate baths (Fisher, Thiebee and Maxted, *Z. anorg. Chem.*, 1910, **67**, 347 ; Blum, Liscomb, Jencks and Baily, *Trans. Amer. Electrochem. Soc.*, 1919, **34**, 101 ; Blum and Haring, *ibid.*, 1921, **40**, 287 ; Betts, "Lead Refining by Electrolysis", New York, 1908 ; Mathers, *Chem. Z.*, 1910, **34**, 1316, 1350 ; *Trans. Amer. Electrochem. Soc* , 1914 **17**, 261) which admit of high current densities and give satisfactorily dense deposits. The principal drawback, however, is that the cost of maintaining these baths is high and at times almost prohibitive. Any reduction in the concentration of the acids leads to the so-called ' *tree* ' formation which is a defect of frequent occurrence in lead deposits produced electrolytically. With the exception of the pioneering work of Glaser (*Z. Electrochem.*, 1900, **7**, 383) and others who have used plumbates, double cyanides, and other alkaline solutions, our knowledge regarding utilisation of alkaline baths for lead plating is meagre. The present work was undertaken with a view to study the conditions of lead deposition using moderately concentrated solutions of litharge, a material which is by no means expensive, in aqueous alkali. The effect of the following factors has been studied : the current density, temperature, separation, (*i.e.* inter-electrode distance), influence of addition agents such as gelatin, antimony and thallium salts and lastly intermediate coating of metallic antimony.

Experimental.

The main composition of the electrolytic bath used in the following experiments was 9·38 g. of litharge (PbO) and 43·5 g. of caustic soda per litre of the aqueous solution. The condition of the electrode surface prior to electrolysis was found to be an important factor. The anode was always a well-cleaned plate of lead of size 6 × 3 cm. approximately. A thin iron plate (7 × 5 cm.) served as the cathode. It was first rendered free from grease by keeping it in a 10 % caustic soda solution for half an hour (*vide infra*) and then washing it with water ; it was next immersed in 1 : 1 hydrochloric acid to remove surface scales and rust, well washed with water and rubbed with sand, when a bright surface was obtained ; it was

finally washed with distilled water and introduced into the bath solutions. It was further cleaned by '*pickling*' with moderately concentrated nitric acid. While this greatly brightened the surface, its activity as an electrode was found to be low, presumably due to surface '*passivation*'.

The electrolysis was carried out in a small glass trough fitted inside another containing water and the whole was immersed in a water-bath, during the experiments on the influence of temperature. The trough was fitted with a wooden disc containing slits at appropriate distances through which the electrodes were introduced. In each experiment about 500 c.c. of the plating solution were used. The change in the strength of the solution during etectrolysis was small, since very low current densities were used. The solution was filtered after each experiment and was used subsequently in a certain set of experiments. Current was obtained from a storage cell; the circuit included a voltmeter, connected across the electrodes in the bath, a sliding resistance and a precision type milliammeter. Results given in Table I refer to current densities varying from 0·100 to 0·668 amp. per sq. dcm. passed during ½ hr. to 3½ hrs. in baths some of which contained a couple of drops of a freshly prepared dilute solution of gelatin in water. Table II shows the influence of temperature on lead plating in the range 17°-70°.

TABLE I.

P. D. = 0·2 volt. Temp. = 18°. Inter-electrode distance = 3 cm.
Cathode = 5 cm. × 3 cm.

No.	*I.*	C. D.	Time.	Addition agent.	Remarks.
1.	15 m. amp.	0·100 amp./dcm².	45 min.	..	Density of deposit very poor.
2.	18	0·120	1 hr.	...	" " "
3.	25	0·166	1 hr.	...	" " "
4	30	0·200	1 hr.	...	" " "
5.	15	0·100	1 hr.	2 drops of gelatin	Initial '*pickling*' with nitric acid deleted. Deposit coherent and greyish white; tended to improve by increasing C D.
6.	20	0·132	1 hr.	"	" " "
7.	25	0·166	1 hr.	"	" " "
8.	30	0·200	3½ hr.	"	" " "
9.	50	0·334	2 hr.	"	Not '*pickled*' with nitric acid P. D. raised to 0·25 volt. "*Tree*" formation sets in which increased with C D.
10.	60	0·400	2 hr.	"	" " "
11.	65	0·434	½ hr.	"	" " "
12.	1000	0·666	½ hr.	"	A central cathode (not '*pickled*') was used between two anodes. Separation between cathode and either anode 2 cm. The deposit was good.

TABLE II.

P. D. = 0·3 volt. C. D. = 0·666 amp./dcm.^{2} Inter-electrode distance = 2½ cm. Size of plates = 5 cm. × 3 cm.

No.	Time.	Temp.	Addition Agent	Remarks.
1.	1 hr.	20°	Two drops of gelation solution.	Deposit tended to "*peel*"
2.	2 hr.	30°	" " "	" "
3.	2 hr.	40°	" " "	Deposit more firm and dense,
4.	2 hr.	50°	" " "	"
5.	½ hr.	50°	A small pinch of antimony oxide is also added.	Deposit poor, "*tree*" formation sets in early.
6.	1 hr.	17°	Four drops. of 1% tartar emetic soln. also added.	"*Free*" formation absent; deposit improved.
7.	1 hr.	22°	" " "	Further improvement in the deposit.
8.	1½ hr.	35°	" " "	" " "
9.	1 hr.	60°	Two more drops of tartar emetic solution added.	"*Treeing*" after an hour; deposit to *peel* off at the edges where "*treeing*" was maximum.
10.	1 hr.	70°-72°	" " "	"Treeing" commenced in about 3/4 hour.

RESULTS.

It is seen from the results in Table I that at a constant bath temperature, an increase in the current density (C. D.) improves the quality of the deposit. There is, however, an optinum value at about 0·334 amp/sq. dcm. (*I*, current used = 50 milliamps.), any further increase in C. D. induces '*treeing* and looseness of deposit. A much higher C. D. could, however, be employed with distinct advantage with a cathode midway between two anodes and a smaller separation. It was observed that the cathodic deposit was almost entirely restricted to the side opposite to the anode. The denseness as also the total amount of the deposit increased when the cathode was rotated so as to expose either of its sides towards the anode. This was

particularly so when placed in the centre (No. 12). These experiments also showed that the addition of a few drops of a dilute solution of gelatin in water had a decisive influence in improving the deposit. Partly similar results were obtained by Mathers (*loc. cit.*) who found that the addition of very small amounts of peptone and olive oil yielded fine grained lead deposits directly on steel surfaces from fluoborate baths which are perhaps the most convenient and economic amongst the acidic baths for lead plating.

It was also observed that the coating was better if the bath was agitated during electrolysis by carefully bubbling a stream of air. This was adopted in all subsequent experiments. Table II shows the influence of temperature on the electrodeposition. It is seen that at constant current density the quality of the deposit improves by increasing the temperature upto a certain extent (about 50°) and deteriorates at higher temperatures. It was also observed during these experiments that the addition of only a few drops of a dilute emetic solution of tartar had a very beneficial effect on the growth of the lead deposit. It is important that the addition agent should be well miscible with the plating solution. It was observed, for instance, that despite its antimony content (No. 5) the addition of a small pinch of antimony oxide was of no avail in reducing the '*tree*' formation. Experiments were also made under similar conditions with potassium tartrate and gelatin as addition agents (Nos. 9, 10). *Treeing* set in fairly soon after the commencement of electrolysis. This shows that the efficacy observed in the case of tartar emetic is to be attributed principally to the dissolved antimony in the bath. Evidence has been adduced by Joshi and Padmanabhan (*J. Indian Chem. Soc.*, 1938, **15**, 178) in that an addition agent influences appreciably the electrodeposit by increasing the rate of *nuclei* formation precedent to the crystal growth on the electrode. In view of the wide range of composition and ease with which antimony and lead alloy together, it was thought that a preliminary coating of antimony on the cathode might yield a denser and an unpealable deposit of lead. Results similar to this have been obtained by A. G. Betts (*loc. cit.*) in lead deposition from fluosilicate baths for plating storage battery fittings, where a preliminary deposit of copper has been found necessary.

In order to produce a preliminary coating of antimony, the need was observed for a yet cleaner and more burnished cathode surface. '*Pickling*' with concentrated nitric acid imparted an apparently increased brightness, but the method was not used for reasons indicated already. '*Electrolytic pickling*' gave very satisfactory results. The iron plate was made the cathode in a bath of hot dilute (6*N*) sulphuric acid, which was electrolysed for about ha

an hour. It was then carefully washed with water and electrolysed for about an hour as a cathode, in an *alkali bath* containing sodium carbonate (43 g.), caustic soda (17 g.), sodium phosphate (13 g.,) per litre of aqueous solution. The plate was then well washed with distilled water and *'pickled'* for a few minutes in dilute sulphuric acid (1 part of acid to 15 parts water by weight). It was washed with boiling water and then dipped in a mixture containing (by weight) concentrated nitric acid (75 parts), concentrated sulphuric acid (100 parts), and sodium chloride (1 part), for a few seconds, and finally well washed with hot water (*cf.* " Theoretical and Applied Electrochemistry " by Thomson, p. 181). It was made the cathode and was electrolysed at 220 in a small bath containing 1·5% tartar emetic solution with a drop of gelatin, which gave the necessary preliminary coating of antimony in about 1¾ hour. This piece was next made the cathode in a lead plating solution (0·25 volt. 100 milliamp. 0·667 amp. per sq. dcm separation 2½-3 hours, 60°.) This gave lead coats which were firmer and of greater thickness than those obtained during any of the previous experiments. Furthermore, it was observed that the addition of small amounts of gelatin and tartar emetic and interrupting the electrolysis for a brief time after every half an hour improved greatly the quality of the cathode deposit and eliminated *tree ing.* Very satisfactory results were also obtained by the use of small quantities of solutions of *thallium carbonate.*

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ELECTRO-CHEMISTRY SECTION,
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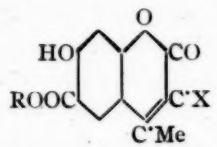
PECHMANN'S CONDENSATION OF METHYL β-RESORCYLATE WITH ETHYL α-ALKYLACETOACETATES.

By S M. Sethna and R. C. Shah.

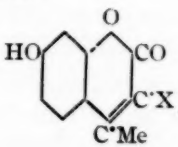
Methyl β-resorcylate has been condensed with ethyl α-methyl-, α-ethyl-, α-propyl-, α-butyl-, and α-benzyl acetoacetates in the presence of sulphuric acid. In each case the condensation product is a mixture of 7-hydroxy-3-alkyl-4-methylcoumarin-6-carboxylic acid and its methyl ester

In continuation of the previous work (*J. Indian Chem. Soc.*, 1937, **14**, 717) we have successfully condensed methyl β-resorcylate with ethyl α-methyl-, α-ethyl-, α-propyl-, α-butyl-, and α-benzylacetoacetates. In each case the condensation product is a mixture of 7-hydroxy-3-alkyl-4-methylcoumarin-6-carboxylic acid and its methyl ester (I, R=H or Me respectively and X = CH_3, C_2H_5, C_3H_7, C_4H_9 and $CH_2{\cdot}C_6H_5$ respectively). The constitution of all the condensation products has been established by decarboxylating the acids by heating in sealed tube with water. The decarboxylated products are directly compared with specimens of 7-hydroxy-3-alkyl-4-methylcoumarins (II, X=CH_3, C_2H_5, C_3H_7, C_4H_9 or $CH_2{\cdot}C_6H_5$ respectively) prepared from resorcinol and ethyl α-alkylacetoacetates. Attempts to condense β-resorcylic acid itself with ethyl α-methylacetoacetate were unsuccessful.

The inhibiting effect of the 4-carbomethoxy group in the resorcinol nucleus on the coumarin condensation is thus found to be less than that of the 4-nitro group, as 4-nitroresorcinol has been found to condense only with ethyl acetoacetate and no product could be isolated by reacting 4-nitro-resorcinol with ethyl α-methyl- or α-ethylacetoacetates (Chakravarti and Banerjee, *J. Indian Chem. Soc.*, 1937, **14**, 37).



(I)



(II)

EXPERIMENTAL.

Purification of Materials.—Ethyl α-ethyl-, α-propyl-, and α-butyl-acetoacetates obtained from Dr. Theodor Schüchardt were found to contain ethyl acetoacetate in appreciable quantity. Ethyl α-butylacetoacetate was purified by distillation under reduced pressure but ethyl α-ethyl- and ethyl α-propyl-

acetoacetates could not be thus purified. When unpurified ethyl α-ethylacetoacetate was condensed with methyl β-resorcylate, the product obtained gave a melting point range of about 30°. On purification by crystallisation it gave methyl 7-hydroxy-4-methylcoumarin-6-carboxylate. The impure ethyl α-ethylacetoacetate was purified by washing repeatedly with 3% sodium hydroxide and then distilled when almost all the ethyl acetoacetate was removed. Ethyl α-propylacetoacetate could not be completely purified by this treatment and on condensation with methyl β-resorcylate it gave a product, melting indefinitely with a long range, which could not be purified by further crystallisation. The acetyl derivative, however, came out pure and the pure coumarin ester (I, R=Me; X=C_3H_7) was obtained from this by hydrolysis.

All the coumarin esters and acids gave an intense violet colouration with alcoholic ferric chloride and a strong blue fluorescence with alkali.

Methyl 7-Hydroxy-3:4-dimethylcoumarin-6-carboxylate (I, R=Me; X=Me).—Sulphuric acid (80%; 60 c.c.) was added gradually with shaking to a mixture of methyl β-resorcylate (15 g.) and ethyl α-methylacetoacetate (14 g.). After keeping for 40 hours at room temperature the reaction mixture was poured over crushed ice. The product obtained was treated with sodium bicarbonate solution. The insoluble solid was filtered, and the filtrate acidified, when the free acid was precipitated as a granular solid. The coumarin ester crystallised from rectified spirit in shining needles, m.p. 212-13°, yield 4·5 g. (Found: C, 63·1; H, 4·8. $C_{13}H_{12}O_5$ requires C, 62·9; H, 4·8 per cent).

It is sparingly soluble in 10% caustic soda solution, rectified spirit, methyl alcohol and petroleum ether and more soluble in benzene, chloroform, acetone and glacial acetic acid.

The acetyl derivative, prepared by refluxing the ester (0·5 g.) for 3 hours with acetic anhydride (2 c.c.) and 4-5 drops of pyridine, crystallised from dilute alcohol in short shining needles, m.p. 178-80°. (Found: C, 62·0; H, 4·8. $C_{15}H_{14}O_6$ requires C, 62·2; H, 4·8 per cent).

The benzoyl derivative, prepared by heating the ester (0·5 g.), dissolved in pyridine, with benzoyl chloride (1·5 c.c.) for 2 hours on a boiling water-bath, crystallised from rectified spirit in long shining needles, m.p. 159-60°. (Found: C, 68·0; H, 4·6. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·5 per cent).

The methyl ether, prepared by refluxing the ester (0·5 g.), dissolved in acetone (50 c.c.) for 20 hours with fused potassium carbonate (1 g.) and methyl iodide (3 c.c.), was crystallised from dilute alcohol in needles, m.p. 185-87°. (Found: C, 63·7; H, 5·3. $C_{14}H_{14}O_5$ requires C, 64·1; H, 5·3 per cent).

7-Hydroxy-3:4-dimethylcoumarin-6-carboxylic acid (I, R=H and X=Me), obtained by the acidification of sodium bicarbonate solution, as mentioned above, was crystallised from rectified spirit in pointed needles, m.p. 263°, yield 1·5 g. (Found : C, 57·7 ; H, 4·7. $C_{12}H_{10}O_5$, $1H_2O$ requires C, 57·2 ; H, 4·8 per cent). The same acid was also obtained by the hydrolysis of the coumarin ester by cold alkali. The ester (0·5 g.) was shaken up with sodium hydroxide (10%, 20 c.c.) and kept for 48 hours when it went completely into solution. The product obtained on acidification was crystallised from rectified spirit in shining needles, m.p. and mixed m.p. with acid obtained from above was 263°, yield 0·35 g.

7-Hydroxy-3 : 4 dimethylcoumarin.—(*a*) The acid (0·3 g.) was heated in a sealed tube with water (25 c.c.) and concentrated hydrochloric acid (5 c.c.) at 180-190° for 7 hours. The product obtained was crystallised from dilute alcohol in faint brown coloured needles, m.p. 260-62°, mixed m.p. with an authentic specimen of 7-hydroxy-3 : 4-dimethylcoumarin from resorcinol and ethyl α-methylacetoacetate was not depressed. Pechmann and Duisberg (*Ber.*, 1883, **16**, 2127) and Chakravarti (*J. Indian Chem. Soc.*, 1931, **8**, 132) give m.p. 256° but Simonis and Remmert (*Ber.*, 1914, **47**, 2231) give m.p. 262°. (*b*) The acid was also conveniently and quantitatively decarboxylated by heating it in a test-tube in an oil-bath at 265-270° till it melted completely and the effervescence ceased.

Methyl 7-hydroxy-3-ethyl-4-methylcoumarin-6-carboxylate (I, R=Me ; X=C_2H_5) was prepared by keeping a mixture of methyl β-resorcylate (8 g.) and purified ethyl α-ethylacetoacetate (10 g.) with sulphuric acid (80% ; 60 c.c.) for 24 hours. The reaction mixture was worked up as before. The ester crystallised from rectified spirit as colourless needles, m.p. 144-46° (Found : C, 63·6 ; H, 5·2. $C_{14}H_{14}O_5$ requires C, 64·1 ; H, 5·3 per cent).

The acetyl derivative, prepared by refluxing the ester with sodium acetate and acetic anhydride, crystallised from dilute alcohol in clusters of tiny needles, m.p 146-47°. (Found : C, 63·1 ; H, 5·2. $C_{16}H_{16}O_6$ requires C, 63·2 ; H, 5·3 per cent).

7-Hydroxy-3-ethyl-4-methylcoumarin-6-carboxylic acid was obtained by keeping the ester (0·1 g.) with sodium hydroxide (5%, 10 c.c.) for 24 hours. The acid obtained on acidification was crystallised from dilute alcohol in needles, m.p. 243-45°. (Found : C, 62·6 ; H, 5·1 $C_{13}H_{12}O_5$ requires C, 62·8 ; H, 4·8 per cent).

The acid was decarboxylated by heating with water in a sealed tube as before. The product crystallised from dilute alcohol in needles, m.p. 196-97°, mixed m.p. with an authentic specimen of 7-hydroxy-3-ethyl-

4-methylcoumarin was not depressed. Chakravarti (*loc. cit.*) gives m.p. 194-98°.

Methyl 7-hydroxy-3-propyl-4-methylcoumarin-6-carboxylate (I, R = Me ; X = C_3H_7).—A mixture of methyl β-resorcylate (15 g.), ethyl α-propyl-acetoacetate (15 g.) and sulphuric acid (80%, 100 c.c.) was kept for 24 hours and then added to water. The pasty mass obtained solidified partially on keeping in a frigidaire for 2 days. It was filtered, dried, washed with light petroleum ether and washed with sodium bicarbonate solution (A). The insoluble portion was crystallised first from rectified spirit and then from a mixture of benzene and petroleum ether, m.p. 133-150°. The product could not be further purified by crystallisation so the pure ester was obtained by the hydrolysis of the acetyl derivative prepared from this impure product. *The acetyl derivative* (0·7 g.) was shaken up with sodium hydroxide (2%, 15 c.c.) in a shaking machine for 2 hours and then kept overnight. The dissolved portion was filtered (B) and reprecipitated with hydrochloric acid. The product obtained was treated with sodium bicarbonate solution to remove the coumarin acid formed and the insoluble portion crystallised from rectified spirit in clusters of tiny needles, m.p. 142-44°. (Found : C, 65·1 ; H, 6·0. $C_{15}H_{16}O_5$ requires C, 65·2 ; H, 5·8 per cent).

The acetyl derivative, prepared as usual, was crystallised from 60% alcohol in long colourless needles, m.p. 113°. (Found : C, 64·0 ; H, 5·6. $C_{17}H_{18}O_6$ requires C, 64·2 ; H, 5·7 per cent).

The methyl ether, prepared as in the previous case, crystallised from 60% alcohol in small needles, m.p. 138-40°. (Found : C, 66·4 ; H, 6·4. $C_{16}H_{18}O_5$ requires C, 66·2 ; H, 6·2 per cent).

7-Hydroxy-3-propyl-4-methylcoumarin-6-carboxylic Acid.—The sodium bicarbonate solution (A) was acidified and the product obtained crystallised from rectified spirit in long colourless needles, m.p. 230-31°. (Found : C, 64·1 ; H, 5·3. $C_{14}H_{14}O_5$ requires C, 64·1 ; H, 5·3 per cent).

The sodium bicarbonate solution (B) gave the same acid as above, m.p. and mixed m.p. with it was the same. The acid was decarboxylated as before and the product obtained crystallised from dilute alcohol in shining needles, m.p. 171-73°, mixed m.p with an authentic specimen of 7-hydroxy-3-propyl-4-methylcoumarin was the same. Chakravarti (*loc. cit.*) gives m.p. 169-71°.

Methyl 7-Hydroxy-3-butyl-4-methylcoumarin-6-carboxylate (I, R = Me ; X = C_4H_7).—To a mixture of methyl β-resorcylate (20 g.) and ethyl α-butyl-acetoacetate (22 g.) sulphuric acid (80%, 130 c.c.) was added gradually with shaking. After keeping for 24 hours the reaction mixture was added

to water. It was worked up as in the case of the propyl analogue and washed with sodium bicarbonate solution. The ester crystallised from rectified spirit in long slender needles, m.p. 163-65°. (Found : C, 66·5 ; H, 6·1. $C_{16}H_{18}O_5$ requires C, 66·2 ; H, 6·2 per cent).

The acetyl derivative, prepared as usual, was crystallised from rectified spirit in long shining needles, m.p. 111-13°. (Found : C, 65·0 ; H, 6·0. $C_{18}H_{20}O_6$ requires C, 65·1 ; H, 6·0 per cent).

The methyl ether, prepared as usual, crystallised from dilute alcohol in light silky needles, m.p. 150-152°. (Found : C, 67·1 ; H, 6·7. $C_{17}H_{20}O_5$ requires C, 67·1 ; H, 6·6 per cent).

7-Hydroxy-3-butyl-4-methylcoumarin-6-carboxylic Acid.—The sodium bicarbonate solution from above on acidification gave the acid which crystallised from rectified spirit in colourless shining needles, m.p. 222°. (Found: C, 65·3 ; H, 5·8. $C_{15}H_{16}O_5$ requires C, 65·2 ; H, 5·8 per cent). The same acid was obtained on hydrolysis of the ester (0·3 g.) by keeping it with cold sodium hydroxide solution (20%, 15 c.c.) for 60 hours.

7-Hydroxy-3-butyl-4-methylcoumarin, obtained by decarboxylation as usual, was crystallised from dilute alcohol (charcoal) in shining plates, m.p. 134-36°. Mixed m. p. with a specimen of 7-hydroxy-3-butyl-4-methyl coumarin prepared from resorcinol (3 g.), ethyl α-butyl-acetoacetate (5·1 g.) and sulphuric acid (80%, 10 c.c.) was the same. (Found: C, 72·7; H,7·3. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·9 per cent).

Methyl 7-hydroxy-3-benzyl-4-methylcoumarin-6 carboxylate (I,R = Me; X = $CH_2 \cdot C_6H_5$)—To a mixture of methyl β-resorcylate (10 g.) and ethyl α-benzylacetoacetate (13·5 g.) sulphuric acid (80%, 60 c.c.) was added with shaking and after keeping for 20 hours the reaction mixture was worked up as before. The ester crystallised from rectified spirit in tiny shining needles, m.p. 186-88°. (Found: C, 70·0; H, 5·2. $C_{19}H_{16}O_5$ requires C, 70·4; H, 4·9 per cent).

The methyl ether, prepared as usual, crystallised from rectified spirit in long colourless needles, m.p. 146-48°. (Found: C, 71·2; H, 5·4. $C_{20}H_{18}O_5$ requires C, 71·0; H, 5·3 per cent).

The acetyl derivative, prepared as usual, crystallised from rectified spirit in tiny needles, m.p. 132-34°. (Found : C, 68·9 ; H, 5·0. $C_{21}H_{18}O_6$ requires C, 68·9; H, 4·9 per cent).

7-Hydroxy-3-benzyl-4-methylcoumarin-6-carboxylic acid was obtained by the hydrolysis of the ester (0·3 g.) by keeping it in contact with cold sodium hydroxide (10%, 30 c. c.) for 60-65 hours. It crystallised from rectified spirit in tiny needles, m.p. 247-48°. (Found: C 69·9; H, 4·7. $C_{18}H_{14}O_5$ requires C, 69·7; H, 4·5 per cent). The same acid was also

obtained by heating the ester (0·3 g.) with sodium hydroxide solution (10%; 25 c c.) in a boiling water-bath for 20 minutes.

The acid was decarboxylated by heating with water in a sealed tube as usual. The product obtained crystallised from dilute alcohol in rectangular prisms, m.p. and mixed m.p. with an authentic specimen of 7-hydroxy-3-benzyl-4-methylcoumarin was 224°. Chakravarti (*loc. cit.*) gives m.p. 224°.

All the analyses recorded are micro-analyses by Dr. Schöeller.

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CHEMICAL EXAMINATION OF THE OIL FROM THE SEEDS OF *SANTALUM ALBUM* (LINN).*

By M. K. Madhuranath and B. L. Manjunath.

Physical and chemical constants of various constituents of the oil from the seeds of *Santalum album* have been determined. The solid has been found to consist of one component with a trace of palmitic acid and from analysis it is considered to be a triolefinic compound of the formula $C_{18}H_{30}O_2$.

Sandal seeds contain about 50% of a valuable highly viscous fatty oil and the problem of its large scale expression from seeds has been recently solved by Mr. Gopalaswamy Reddy of New Oil Mills, Bangalore. The chief difficulty in large scale production consists in the high cost of the collection of the seeds. The oil itself has been the subject of several preliminary investigations (K.A.N. Rao *et al*, *Quarterly J. of the Mysore Forest Dept.*, 1934, p. 7; Iyer, *Analyst*, 1935, **60** 319; Sreenivasaya and Narayana, *J. Indian Inst. Sci.*, 1936, **19A**, 1). In view of the possibilities of the commercial exploitation of the oil, a detailed investigaiion of the various constituents was undertaken with the following results.

Experimental.

The oil was prepared by the extraction of the seeds (oil content 44%) by means of petroleum ether (b.p. 60-70°). The following table gives the physical and chemical constants of the oil thus obtained and of the oil obtained by the expression of the seeds.

Table I.

	Extracted oil.	Expressed oil
Colour	Golden yellow	Dark brown
Specific gravity	0·9356 at 25°/25°	0·9346 at 27°/27°
Refractive index	1·4891 at 30°	1·4884 at 30°
Saponification value	176	176
Iodine (Hanus) ,,	153	153
Thiocyanogen ,,	151	—
Acid ,,	29	44
Reichert-Meisel ,,	0·9	0·8
Acetyl ,,	22	25
Maleic anhydride ,,	3·9	—
Unsaponifiable matter	8·8%	13%
Viscosity	243 at 50°/50°	

* Part of the thesis submitted by M. K. Madhuranath for the M.Sc. Degree of the Mysore University.

The maximum absorption of oxygen by the extracted oil, when exposed in thin layers in an atmosphere of oxygen, was found to be 13%. On drying the oil was converted into an elastic film.

The oil was saponified by alcoholic potash when an elastic white sticky material separated out (5·2 g. from 100 g. of oil). This was removed by filtration through a thick cloth. The fatty acids were liberated from the soap solution and worked up in the usual manner.

TABLE II.

	Total fatty acids.	Solid acids.	Liquid acids.
Percentage	87·2	51	49
Mean M. W.	291	288·6	301·6
Iodine value (Hanus)	111	119·0	108
Resin acids	2·3%	—	—

Liquid Acids.—These were purified by the distillation of their methyl esters in vacuum and the liberated acids were examined by the method of oxidation (Lapworth and Mottram, *J. Chem. Soc.*, 1925, 1628) and bromination (Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes", 6th Ed., Vol. I, 585). In the former case, only dihydroxystearic acid (m.p. 130°; M.W., 315·4) could be isolated. When the products of bromination were dissolved in cold petroleum ether a small amount (0·08 g. from 10 g. of liquid acids) of hexabromostearic acid (m.p. 183; M.W., 762) separated.

These results indicate that the liquid acids consist mainly of oleic acids together with a small amount of linolenic acid.

Solid Acids.—The acids obtained from the insoluble lead salts during the Twitchell separation were esterified with methyl alcohol and the esters (220 g.) were fractionally distilled at 1 mm. pressure.

TABLE III.

No. of fraction.	Range of temperature.	Weight of fraction.	Mean M W. of acids from saponification value.
1	Up to 180°	0·5 g.	...
2	180-183°	140	275·8
3	183-188°	40	278·8
4	188-190°	8	274·1
5	190-194°	17	271·0
6 Residue and loss		9·5	...

From the first fraction a small amount (0·1 g.) of pure palmitic acid was isolated (m. p. 61°; M. W. 255), but no pure material could be obtained from the residue which was highly resinous.

Fractions 2—5 were very carefully refractionated at a pressure of 0·6 mm. Most of the material distilled over between 172-175° and only very small amounts were collected between 175-180° and above 180°. Here also there was considerable resinification.

TABLE IV.

	Range of fraction.	Mean M. W. of acids from saponification value.	Iodine value (Hanus).
A	172-175°	274·3	133
B	175-180°	275·3	133
C	Above 180°	278·4	131

The results indicate that the solid acids consist of one component with a trace of palmitic acid. The acid liberated from the refractionated esters solidified gradually and on recrystallisation from petroleum ether or methyl alcohol melted at 41-42°. (Found: C, 77·8, 77·8; H, 11·0, 11·0; M. W., 281, 281. $C_{18}H_{30}O_2$ requires C, 77·7; H, 10·8 per cent. and M. W., 278). It is very soluble in all the usual organic solvents and possesses a characteristic odour. Its *p*-phenylphenacyl ester melts at 56-57°.

Analysis indicated that the acid was a triolefinic compound of the formula $C_{18}H_{30}O_2$. Although iodine value obtained was 133 against 274 for a pure trienic acid of the formula $C_{18}H_{30}O_2$, it may be due to the difficulty of complete iodine addition. But when it was subjected to quantitative catalytic hydrogenation it took up 3·1 and 3·0 mols. of hydrogen and the product formed was found to be stearic acid (m. p. 69-70°; M. W., 286; m. p. of *p*-bromophenacyl ester, 90°). The thiocyanogen number of the pure acid was 130 and the maleic anhydride number, 8·4 (Ellis and Jones, *Analyst*, 1936, **61**, 812). It did not give any crystallisable material on bromination carried out as with liquid acids. Attempts to prepare a crystallisable adduct with maleic anhydride (Kaufmann and Baltes, *Ber.*, 1936, **69**, 2679) were also unsuccessful.

The acid was also found to be different from punicic acid (m. p. 44°, Toyama and Tsuchiya, *J. Soc. Chem. Ind. Japan*, 1935, **38**, 182 B, 185 B;

Farmer and Henvel, *J. Chem. Soc.*, 1936, 1809) since it could not be made to undergo photoisomerisation into β-elaeostearic acid. There is every indication that it is a new trienic acid and the name *santalbic acid* is proposed for it. A study of its constitution is in progress.

Unsaponifiable Matter.—A small amount of phytosterol (m. p. 131°; m. p. of acetate, 118°) was isolated from the ether extract of the sodium soap.

We are indebted to Mr. P. R. Krishnaswamy for the constants of the expressed oil and for assistance during the course of this work, and to Mr. H. S. Narayana Rao for the supply of sandal seeds.

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ROTTLERIN. PART II.

By Kartar S. Narang, Jnanendra N. Ray and Bharpur Singh Roy.

The N_2O_3 adduct of rottlerin pentamethyl ether is catalytically reduced to a hydro derivative and also isomerises with alkali to a substance giving strong violet ferric reaction. These products are discussed in the light of Robertson's latest formula for rottlerin. It is concluded that the formula of Robertson *et al* does not satisfactorily explain all the known facts about rottlerin.

In Part I (*J. Chem. Soc.*, 1937, 1862) we advocated that rottlerin is $C_{27}H_{26}O_7$. Since then we have amended this view (*Current Sci.*, 1938, **6**, 333 ; *Chem. Ind.*, 1938, **57**, 134) on account of the fact that the oxide described in Part I and nitrous acid addition product are better explained on the basis of a $C_{31}H_{30}O_8$ formula containing five hydroxyl groups.

When the methyl ether of rottlerin (17 g.) is treated with sodium nitrite under the conditions described in experimental sequel a yield of 15·5 g. of the adduct is obtained. This indicates that the product is a simple N_2O_3 addition product and no degradation of the molecule has taken place.

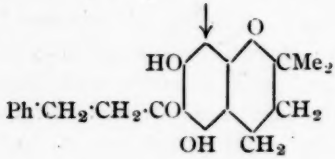
The N_2O_3 adduct (m. p. 209°, decomp.), isomerises to a substance, m.p. 152-53°, by alcoholic potassium hydroxide. This isomeric substance is soluble in alkali. The dihydro derivative of the nitrosite prepared by the catalytic reduction of the substance, m. p. 209°, also isomerises with alkali to a substance, m. p. 139°. This substance is also formed when the *iso*nitrosite, m. p. 153°, is catalytically reduced. The nitrosite, m. p. 209°, can be methylated with alkali and dimethyl sulphate to give a substance, m. p. 192-93°, which may be a mono- or a dimethyl ether of the original product, m. p. 209°. In this connection we wish to state that both the *iso*nitrosite and the dihydro-*iso*nitrosite give deep violet colour with ferric chloride. The nitrosite, on the other hand, shows no ferric reaction and is insoluble in cold aqueous alkali. Therefore, the methylation takes place *via* the *iso*nitroso stage. To prove this, the *iso*nitrosite was independently methylated and the same product (m. p. 193°) was isolated. We attempted to hydrolyse the *iso*nitrosite with formaldehyde and alcoholic hydrochloric acid but we were unable to isolate any definite product. We are engaged in bringing about this hydrolysis by other methods.

The *iso*nitrosite is not a simple hydrolytic product of the nitrosite as it is unaffected when kept in contact with phosphoryl chloride for 2 hours. If the *iso*nitrosite formation was due to simple addition of water we would

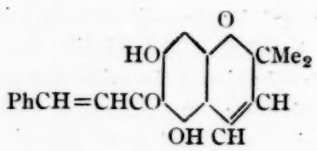
expect that *iso*nitrosite would be retransformed into the nitrosite under these conditions. If the *iso*nitrosite is the oximino form of the nitroso grouping, then also we would expect a Beckmann transformation.

The dihydronitrosite, m. p. 163°, on oxidation with potassium permanganate in acetone solution furnishes a substance crystallising in pale yellow needless m. p. 124°, to which we have provisionally assigned the formula $C_{26}H_{29}O_8N$. It seems that this product is formed by the elimination of a fragment of the original rottlerin molecule.

In Part I (*loc. cit.*) we described a hydrolytic product ($C_{20}H_{22}O_4$) of tetrahydrorottlerin obtained with alcoholic hydrochloric acid. We suggested that this product may be identical with the product obtained by Robertson *et al* (*J. Chem. Soc*, 1937, 748) by the hydrolysis of tetrahydrorottlerin with baryta. We have now methylated our product and found it to be identical with dimethyltetrahydrorottlerone described by Robertson *et al* (*loc. cit.*). We obtained a yield of 3·2 g. from 8 g. of tetrahydrorottlerin, whilst Robertson *et al* (*J. Chem. Soc.*, 1938, 311) record a yield of 5-6 g. from 20 g. by alkaline hydrolysis. These authors have advanced the structure (I) for tetrahydrorottlerone mainly on its degradation to β-phenylpropionic acid and 5:7-dihydroxy-2:2-dimethylchroman.



(I)



(II)

We are unable to subscribe to the above view of its structure on the following grounds:—

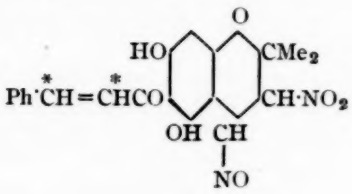
(*a*) The dimethyl ether of tetrahydrorottlerone does not give any indication of the formation of a pyrillium salt when saturated with hydrogen chloride in ether with salicylaldehyde.

(*b*) It has not up to now yielded either a semicarbazone or an oxime.

(*c*) Rottlerone (II), the hydroxychalkone, should isomerise to the corresponding flavanone.

The other half of the rottlerin molecule is supposed to be joined at the place indicated by the arrow in the formula (I). If rottlerone had the structure

(II) then the N_2O_3 adduct would probably have the structure (III) or an alternative with the NO and NO_2 at the positions marked*. It is possible to get benzaldehyde even if the N_2O_3 had added at the positions marked but provisionally we put the groups NO and NO_2 in the chromene ring for several reasons. The nitrosite would be (III) if rottlerin is (II) with the second half attached at the arrow as Robertson *et al* suppose.



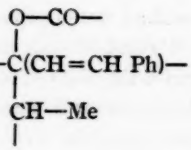
(the position of NO and NO_2 may be reversed.)

(III)

On this basis, the *iso*nitrosite would be the corresponding oximino compound. But since the *iso*nitrosite shows a strong violet ferric reaction, therefore in the formation of the *iso*nitrosite probably a phenolic hydroxyl is set free. We believe that the chroman ring opens up in the *iso*nitrosite formation Moreover, we have noticed that in the hydrolysis of tetrahydrorottlerin with alcoholic hydrochloric acid, if the reaction is stopped after 8 hours very little tetrahydrorottlerone is formed. The product consists mostly of an acid easily soluble in sodium bicarbonate. Unfortunately this acid is unstable and is difficult to purify. However, if this acid be taken in alcohol and heated with hydrochloric acid for a further period of about 16 hours, a good yield of tetrahydrorottlerone is obtained. Therefore rottlerone is formed from rottlerin *via* an acid. Hence it is most probable that in the rottlerin molecule a suppressed carboxyl occurs in the form of an ester or a lactone grouping.

In Robertson's formula of rottlerin there is no place for such a group. We think that the group PhCH=CH should be removed from the phloroglucinol nucleus and be placed near the chromene ring. The formation of a phenolic *iso*nitroso derivative suggests that the oxygen of the chromene ring is set free by alkali and the carboxyl of the lactone relactonises with the hydroxyl of the tertiary carbinol. On this basis several alternative structures for rottlerin can be formulated including one in which one of the methyls of the *gem*-dimethyl group could be attached to a COOH, but the present indications are that the *gem*-dimethyl group exists as such and the lactone is probably attached to the 3:4 carbon atoms of the chromene ring. We think that the methyl phloroglucinol half is joined to

chromene ring through a



The formation of $C_{26}H_{29}O_8N$ by the oxidation of the dihydronitrosite can be explained by the extrusion of C_{10} and a nitrogen atom from the right hand side of chromene ring.

We are engaged in oxidising dimethylrottlerone with lead tetra-acetate and other oxidising agents. Preliminary oxidation with SeO_2 gave a substance in which the double bond containing PhCH was regenerated as the product gave benzaldehyde with alkali.

In criticism of Robertson's latest formula for rottlerin (*J. Chem. Soc.*, 1938, 310) we find the following difficulties :—

(*a*) No derivatives of rottlerin give any reactions of a ketone.

(*b*) No bromoform is formed from rottlerin methyl ether with sodium hypobromite hence there cannot be a $CO{\cdot}CH_3$ group in the molecule.

(*c*) The formation of the benzeneazomethylphloroacetophenone (Brockmann and Maier, *Naturwiss.*, 1937, **25**, 460) is difficult to visualise unless it is assumed that the diazo complex enters the second half of the rottlerin molecule by detaching the rottlerone half. In our opinion the reaction of diazobenzene chloride with rottlerin is comp'ex, the $CO{\cdot}CH_3$ group of Brockmann and Maier's product arises through the hydrolysis of the chromene ring.

Experimental.

Action of Nitrous Acid on Rottlerin Pentamethylether.—Rottlerin methyl ether (17 g.), dissolved in acetic acid (255 c.c.) by gentle warming, was treated with powdered sodium nitrite (17 g. at 30° during ½ hour. The solution was then heated to 80° for 10 minutes and then diluted with a large volume of water (700-800 c.c.). The precipitated bright yellow solid was collected, dried and triturated with ether (about 100 c.c.) in which a small portion dissolved. The crude insoluble material (m.p. 204-6°) weighed 15·5 g. It was crystallised from boiling toluene in pale yellow plates, m.p. 208-9° (decomp.). (Found : C, 63·66, 63·87 ; H, 6·2, 5·9 ; N, 4·2, 4·17. $C_{36}H_{40}O_{11}N_2$ requires C, 63·9 ; H, 5·92 ; N, 4·14 per cent).

Action of Alkali on the Nitrosite : Formation of the iso*Nitrosite.*—The foregoing nitrosite (2 g.) suspended in alcohol (15 c.c.) was treated with 33% aqueous sodium hydroxide solution (10 c.c). The solution was

warmed on the steam-bath for 3 minutes and then the alcohol removed in *vacuo*. The dark red solution was diluted with water (80 c.c.) and extracted with ether to remove neutral impurities. The aqueous portion on acidification with concentrated hydrochloric acid furnished a sticky yellow product which crystallised from alcohol in yellow plates, m.p. 153°, yield 1·3 g. This substance gives a violet ferric reaction. (Found : C, 63·57 ; H, 5·98 ; N, 4·11. $C_{36}H_{40}O_{11}N_2$ requires C, 63·9 ; H, 5·92 ; N, 4·14 per cent).

Methylation of iso*Nitrosite*.—To the foregoing *iso*nitrosite (1 g.) dissolved in methanol (10 c.c.) sodium hydroxide solution (15 c.c. of 80%) and dimethyl sulphate (8 c.c.) were added in small portions alternately. The temperature of the mixture was not allowed to rise. The sticky mass obtained by dilution with water (100 c.c.) was washed with water by decantation and finally crystallised from alcohol in bright yellow plates, m.p. 192-93°, yield 0·6 g. (Found : C, 65·22 ; H, 6·02; N, 4·03. $C_{37}H_{42}O_{11}N_2$ requires C, 64·35 ; H, 6·09 ; N, 4·06. $C_{38}H_{44}O_{11}N_2$ requires C, 64·77 ; H, 6·25 ; N, 3·98 per cent).

Action of Alkali on Dihydronitrosite.—The dihydronitrosite (Narang, Ray and Roy, *J. Chem. Soc.*, 1937, 1864) was treated with alkali as described in the case of the nitrosite except that the alkaline solution was heated on the steam-bath for 10 minutes. The product, isolated as described before, crystallised from alcohol in pale yellow elongated plates, m.p. 139°, yield 2 g. from 2·5 g. of the dihydronitrosite. This substance also gives a deep violet ferric reaction. (Found : C, 63·33 ; H, 6·12 ; N, 4·13. $C_{36}H_{42}O_4N_2$ requires C, 63·72 ; H, 6·19 ; N, 4·13 per cent). This product can be easily obtained by the reduction of the *iso*nitrosite described before in ethyl acetate with palladium and hydrogen. The use of platinic oxide catalyst under atmospheric pressure also furnished the same product and no perhydro derivative or an amine was formed.

Oxidation of the Dihydronitrosite with Acetone-permanganate.—A solution of the dihydronitrosite (2·5 g.) in acetone (40 c.c.) was added in one lot to a solution of potassium permanganate (1·5 g.) in acetone (50 c.c.) and water (25 c.c.). The mixture was shaken for 1½ hours at 45-50°. The filtrate from the manganese oxide was evaporated and the residue was extracted with ether.

The ethereal solution was washed with sodium bicarbonate solution (1%) and water, dried and then evaporated. The residue was crystallised from alcohol (25 c.c.) in long pale yellow needles, m.p. 124°. (Found : C, 64·6 ; H, 6·06 ; N, 2·89. $C_{26}H_{29}O_5N$ requires C, 64·59 ; H, 6·00 ; N, 2·89 per cent).

Tetrahydrorottlerone.—A mixture of tetrahydrorottlerin (8 g.) in alcohol (400 c.c.), hydrochloric acid (*d* 1·14, 80 c.c.) and water (40 c.c.) was heated for 26 hours. The solution was filtered hot and the insoluble powder, thus obtained, was crystallised from ethyl acetate. On heating for a shorter period, an acidic substance was formed which resisted purification. The yield of the crystallised product is 3·2 g. (For analytical data *vide* Narang, Ray and Roy, *loc. cit.*). The substance gives on methylation a methyl ether, m.p. 102°. (*cf.* Robertson *et al*, *J. Chem. Soc.*, 1938, 311).

UNIVERSITY CHEMICAL LABORATORIES,
LAHORE.

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STUDIES IN CATALYTIC DEHYDRATION.

By S. Chatterjee, M. Sanyal and M. Goswami.

Intramolecular catalytic dehydration has been shown to take place between ethers and ammonia in presence of thoria and alumina. The process has been extended to ether and aniline and very good yield of diethylaniline has been obtained.

Intramolecular dehydration has been attempted with numerous organic compounds and various catalysts. The present work is an attempt to prepare secondary amines and tertiary amines from ethers according to the following reactions.

(*i*) $NH_3 + \begin{matrix} R \\ R' \end{matrix}\!\!>\!O \rightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!NH + H_2O$

(*ii*) $R''NH_2 + \begin{matrix} R \\ R' \end{matrix}\!\!>\!O \rightarrow \begin{matrix} R \\ R' \end{matrix}\!\!>\!N - R'' + H_2O$

Such reactions employing ethers do not appear to have been studied. Previous attempts to prepare primary amines have been made by Sabatier and Mailhe (*Compt. rend.*, 1909, **148**, 900; 1911, **153**, 160, 1204) from ammonia and alcohols using thoria as catalyst and by Roy (*J. Indian Chem. Soc.*, 1928, **5**, 383) who obtained a good yield of a mixture of mono- and dimethylaniline from aniline and methyl alcohol using the same catalyst.

We have prepared various aliphatic secondary amines from the corresponding ethers and ammonia. Aromatic ethers like diphenyl oxide and anisole gave indications of the formation of amines but yielded appreciable quantities of unstable white needles which decomposed on exposure into corresponding ether and ammonia. With diethyl ether and aniline using alumina as a catalyst we have been able to get 86% yield of diethylaniline. From the experimental results it would appear that thoria in general is not a good catalyst for intramolecular dehydration between ethers and ammonia or amines.

Experimental.

Procedure.—Thoria was prepared from thorium nitrate by heating to drive off nitrous fumes. Aluminium oxide was prepared from alum by

Tetrahydrorottlerone.—A mixture of tetrahydrorottlerin (8 g.) in alcohol (400 c.c.), hydrochloric acid (*d* 1·14, 80 c.c.) and water (40 c.c.) was heated for 26 hours. The solution was filtered hot and the insoluble powder, thus obtained, was crystallised from ethyl acetate. On heating for a shorter period, an acidic substance was formed which resisted purification. The yield of the crystallised product is 3·2 g. (For analytical data *vide* Narang, Ray and Roy, *loc. cit.*). The substance gives on methylation a methyl ether, m.p. 102°. (*cf.* Robertson *et al*, *J. Chem. Soc.*, 1938, 311).

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Experimental.

Procedure.—Thoria was prepared from thorium nitrate by heating to drive off nitrous fumes. Aluminium oxide was prepared from alum by

precipitating aluminium hydroxide by ammonia, washing the precipitate free from sulphate and ammonia, and igniting it at 150° for several hours. For the preparation of aliphatic amines, ammonia gas, generated by allowing liquor ammonia to drop on to solid sodium hydroxide (stick) and dried by passage through towers containing dry quick lime, soda lime and anhydrous potassium carbonate, was passed through anhydrous ether and the mixed vapour was led over thoria or alumina in a combustion tube heated at the requisite temperature in a combustion furnace. The receiver, connected by an adapter with the combustion tube, was fitted with a condenser cooled by circulation of iced water. For the preparation of diethylaniline, the vapour of anhydrous ether was passed through dry aniline kept near its boiling point and the mixed vapour was treated as above.

Experiments with Aliphatic Ethers and Ammonia.

Higher ethers were prepared by the method of Senderens (*Compt. rend.*, 1924, **179**, 1015). The presence the respective secondary amines was proved by the preparation of picrates and hydrochlorides. The reaction products were dried over quick lime and treated with mercuric oxide to remove traces of ammonia, filtered and analysed. A definite amount of the clear liquid was treated with acetic anhydride and the excess was titrated with $N/10$-baryta solution. The following results were obtained.

Diethyl ether.

Catalyst.	Temperature of reaction.	Yield of diethylamine.
Thoria	275-285°	1·693%
,,	350°	1·263
,,	400°	0·3502
,,	425°	0·6219
Alumina	200°	5·682
,,	250°	6·183
,,	300°	3·996

n- Propyl ether.

Catalyst.	Temperature of reaction.	Yield of dipropylamine.
Thoria	250°	3·747%
,,	300°	8·262
,,	350°	3·788
Alumina	250°	1·574
,,	300°	2·424

n-Butyl ether

Catalyst.	Temperature of reaction.	Yield of dibutylamine.
Thoria	250°	0·4996%
,,	300°	1·057
,,	350°	0·861
Alumina	250°	2·1
,,	300°	0·34

n-Amyl ether.

Catalyst.	Temperature of reaction.	Yield of diamylamine.
Thoria	250°	3·321%
,,	300°	3·633
,,	350°	3·515
Alumina	270°	3·006

Diethyl ether and Aniline.

The reaction product was dehydrated over quick lime and weighed. A portion was diazotised and titrated against standardised R-salt ; another portion was acetylated and excess of acetic anhydride was titrated back with baryta (*cf.* Roy, *loc. cit.*). In this way the percentages of aniline, monoethylaniline and diethylaniline were determined. The following are results with alumina.

Temperature of reaction.	Weight of aniline.	Weight of reaction product.	Aniline.	Mono-ethylaniline.	Diethylaniline.
370-375°	102 g.	96 g.	12%	14%	74%
395-400°	81·6	82	12	17	71
415°	91·8	83	10	25	65

It would appear from the foregoing results that although the yield of tertiary amine was high, there was some decomposition of the product which increased with the rise of temperature.

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DIMETHYLAMINO- AND DIETHYLAMINOPHENYLIMINO-CAMPHORS. REAGENTS FOR MERCURY.

By Mahan Singh.

Ammonium nitrosophenylhydroxylamine (*Bull. soc. chim. Belg.*, 1930, **39**, 170) forms an insoluble compound with mercury and has been used for its estimation. It gives a similar insoluble product with a number of other metals and therefore cannot be regarded as a specific reagent for mercury.

p-Dimethylaminobenzylidene rhodamine (*Monatsh.*, 1905, **26**, 1203) is another reagent used for mercury, silver and copper (ous). Mercurous ions give in neutral or acid solution a brilliant purple colouration. Mercuric ions yield a reddish colour in neutral solution or in the presence of dilute nitric acid. This colour is discharged by hydrochloric acid.

Diphenylcarbazone (*Compt. rend.*, 1908, **146**, 754) also has been used for the detection of mercury, but a number of metals interferes with the detection of small amounts of mercury.

Dimethylamino- and diethylaminophenyliminocamphors (*J. Indian Chem. Soc.*, 1935, **12**, 219, 768) add to the list of the compounds used for the detection of mercury.

The alcoholic solution (1%) of the dimethyl compound gives a deep scarlet colour with a mercuric salt. The reagent does not give any indication with Pb, Cu, Ni, Co, Fe, Ca, Ba, Cd, Mn and Mg salts. Bismuth and silver salts give a faint pink colouration along with a small amount of a white precipitate.

The diethyl compound, however, gives a deep violet colourat on with mercurous and mercuric salts. This colour vanishes when a stream of oxygen is passed into the solution and reappears on adding a small amount of the reagent.

Detection of Mercury by the Spot Reaction.—Filter paper strips are soaked in 0·5% solutions of these compounds and are dried in the atmosphere. A drop of the mercuric salt solution gave a rich pink coloured spot which vanished on exposure to the vapours of ammonia. The depth of the colour is proportional to the amount of mercury present. This reaction is sensitive to one part of $HgCl_2$ in 50,000 parts of water.

A drop of mercurous nitrate solution, however, gave a pink saw-like tinge round a white spot. On exposure to ammonia the spot changed to black. The sensitiveness of this reaction is of the same order.

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Received June 27, 1938.

REVIEWS

Qualitative Inorganic Analysis—By A. J. Berry, M.A., Fellow of Downing College and University Lecturer in Chemistry. Published by the Cambridge University Press, 1938. Pp. 147. Price 6 sh.

This book on qualitative inorganic analysis is evidently intended for those who have already received some preliminary training in the subject, for it omits all descriptions of commoner operations which a beginner must learn before he proceeds with his analytical exercises. Strictly speaking, the book is more a guide than a text-book on qualitative analysis as the discussion on theoretical principles, explanation of the reactions, equations, formulae etc. have been introduced to the extent which may be described as irreducible minimum. Nevertheless it contains all the important and useful tests and reactions of individual elements and acid radicals, including the so-called rare-elements, which, as a departure from the established custom, have been treated along with the commoner elements. Another special feature of the book lies in the introduction of many new organic reagents for selected tests both in the ordinary way as well as in the form of " spot reactions".

The author discourages the traditional tabular methods of presentation and has practically avoided them in his book. That the students place too much reliance upon analytical tables at the neglect of regular study and rational thinking is an experience, common to all teachers of analytical chemistry. Nevertheless, tables are very convenient in presenting one's results in a condensed form.

The book, though written primarily in the interests of the author's own pupils, will still serve as a very useful guide to all students in their laboratory work for the degree course.

There are, however, certain mistakes in the book, which, it is hoped, will be corrected in the next edition. These are: on page 48, under Molybdenum the author states that the carmine red colour produced by the action of ammonium thiocyanate on molybdenum compounds in presence of hydrochloric acid and a reducing agent is due to the formation of ammonium molybdothiocyanate, $(NH_4)_3\,[(Mo(SCN)_6]$. This is not correct. Ammonium molybdothiocyante is yellow. The red colour is due to the pentavalent molybdenum compound $Mo(OH)_2(SCN)_3$.

On p. 90, the equation

$$2HNO_2 = H_2O + NO + N_2O_3$$ is evidently wrong.

On p. 103, in describing the action of potassium cyanide on cupric sulphide the author states that colourless potassium cupricyanide $K_2[Cu(CN)_4]$ is formed. This is wrong ; it should be potassium cuprocyanide $K_3[Cu(CN)_4]$. On p. 111, towards the bottom $Fe'''(C_2O_3)$ should be $Fe'''(C_2O_4)_3$.

P. R.

Micromethods of Quantitative Organic Elementary Analysis —By Joseph B. Niederl and Victor Niederl. Published by John Wiley & Sons Inc. New York And Chapman & Hall, Ltd., London, 1938. Pp. XVI+271.

The authors state in the preface that " this book is primarily in a laboratory manual for teaching and practicing the methods of quantitative organic microanalysis of pure organic compounds under ordinary laboratory conditions and is intended principally for colleges giving a course in this subject and for organic chemical research laboratories, either industrial or academic." In the reviewer's opinion the book meets this object. It is a compilation of up-to-date and carefully selected methods of organic micro-analysis. Although based on Pregl's original methods, certain changes and modifications have been introduced with a view to enable chemistry students without any previous training in microanalytical work to follow the procedures.

The first section (40 pages) of the book describes micro-balance and other equipments and utensils necessary for microanalytical work. The analytical section (pages 125) includes the determinations of metals, residues, neutralization equivalent, nitrogen (Dumas and Kjeldahl), carbon, hydrogen, halogens, sulphur, phosphorous and arsenic. The third section (21 pages) contains a description of four different methods (ebullioscopic, cryoscopic, vaporimetric, and osmotic) of determining molecular weights. This is followed by a section (27 pages) on the determination of important groups, such as alkoxy, alkimido, acetyl and active hydrogen. Literature has been cited extensively and upto 1938. This will be very helpful to those who intend to consult the original literature. The appendix (15 pages) contains many useful informations on such subjects of general interest as teaching of elementary microanalysis, lay-out of the microchemical laboratory and microchemical literature. A special chapter has been devoted to calculations, which will prove very handy to the analysts.

Tables for nitrogen reduction and logarithms (5 figures) are also provided.

The paper, printing and get-up are of superior quality. The authors are to be congratulated for bringing out this excelient volume, which is undoubtedly the most up-to-date and best book on the subject.

P. K. B.

Negative Ions—By H. S. W Massey, Ph.D., Lecturer in Mathematical Physics, Department of Queen's University, Belfast. Published by the Cambridge University Press, 1938. Pp. 104, price 6 sh.

This is one of the first three publications of the new series "Cambridge Physical Tracts" published by the Cambridge University Press. The object of the series, the publishers announce, is to provide authoritative accounts of subjects of topical physical interest by those actively engaged in research. As such we welcome this new contribution to the subject of quantum mechanics in its application to the theory of electric discharge phenomena and to the new "attachment theory" of the ionosphere by one whose name is so well known in this field of investigation.

The title of the work raises the expectation that the book will be found to contain exhaustive discussions on all the aspects of negative ions though, of course, the note by the publishers on the back of the cover warns the reader that his expectations may not be fulfilled. A glance through the book shows that special attention has been paid to the properties and formation of negative ions with reference to the ionospheric problem, and exhaustive study of such ions has been made mainly from this view point.

Starting with a general historical introduction the author shows the advantages of quantum-mechanical treatment of atomic problems and tackles the theory of the formation of negative ions with this method. He, however, points out the great difficulties of applying this method generally, and has not been able to suggest any method which may be effective in getting results of any quantitative significance. The perusal of the book shows the practical limitations of the methods of quantum mechanics. There is no flaw in the theory—the formulae with their qualitative interpretations are very illuminating, but, when one seeks for exact numerical results one is faced with difficulties which, if not insuperable, become an extremely complicated task and one has to be satisfied in most cases with approximations. The author himself ultimately relies on the results of the experiments of such

workers as Loeb, Lozier, Tate etc. He has put together their results in a very useful form and has supplemented them with qualitative but consistent theoretical discussions. The last chapter deals directly with ionospheric problems and is an elaboration of the author's own work o nthesame.

Inspite of the fact that the major portion of the tract treats probl-ms of negative ions with special reference to the ionosphere, the author's statement that nothing important has been left out remains true and the work will be very useful not only to investigators in the field of the new "attachment theory" of the ionosphere but also in other allied subjects.

B. R.

THE CHEMISTRY OF SOME DERIVATIVES OF DECALIN. PART I.

By K. Ganapati.

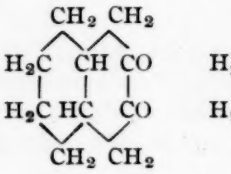
On oxidation with selenium dioxide, *trans*-β-decalone yields a diketone which is proved to be 2 :3-diketo-*trans*-decalin. On monobromination, the decalone yields 2-keto-3-bromo-*trans*-decalin, the bromine atom of which is very inert to the usual reagents. The dibromo compound obtained from decalone, which yields with alkali hexahydrohydrindenol-β-carboxylic acid, is most probably 2-keto-3 :3-dibromo-*trans*-decalin. 2 :3-Diketo-*trans*-decalin yields on reduction (*a*) the corresponding dihydroxy compound, m. p. 141°, with sodium amalgam and (*b*) another isomeric dihydroxy compound, m. p. 126°, with aluminium amalgam. The stereochemistry of the dihydroxy-*trans*-decalins is discussed and it is suggested that the above two compounds have the hydroxy groups in the *trans*-positions.

Wallach and Wissenborn (*Annalen*, 1924, **437**, 153) treated the dibromo compound furnished by the bromination of decalone (which we now consider to be *cis* from its physical constants n_D, 1·49317 and d^{19}, 1·0055) with aqueous potash and obtained (besides other acidic products*) two diketones, m. p. 88-89°, and m. p. 99-100°. The former, which was the main product, was proved by them to be 2:3-diketodecalin (which we now consider to be *cis*), while the latter, obtained in small yields, was not thoroughly investigated. It was suggested to be an isomer (*i.e.* 1:2-diketodecalin) or a "diketone of a lower hydrogenation product." Recently, Rao and Kuppusamy (*J. Annamalai Univ.*, 1937, **7**, 23) oxidised *trans*-β-decalone with selenium dioxide and obtained two fractions: (*i*) b. p. 106-108°/6 mm. and (*ii*) b. p. 126-30°/6 mm. The former was considered by them to be the diketone (1:2 or 2:3) of *trans*-decalin, while the second fraction was not investigated.

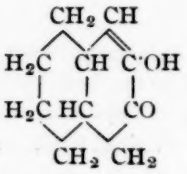
trans-β-Decalone was smoothly oxidised by selenium dioxide in boiling alcohol to yield a crystalline solid, m. p. 99-100°, which has now been proved to be 2:3-diketo-*trans*-decalin (I) by the following properties and reactions: (*i*) it is colourless and forms an enol benzoate and exhibits the properties of the diosphenol form (II) (*cf.* Robinson, "The Electronic Theory of the

* The dibasic acid, $C_{10}H_{16}O_4$, obtained by the authors, is probably *cis*-cyclohexane-1 :2-diacetic acid (m. p. 159-61°); but the high melting point (165°) recorded by them is probably due to an error. It is not likely that this acid can be the *trans* isomer (m. p. 167°) because the starting decalone is predominently *cis* and an isomerisation to *trans* form during the reaction is very remote.

Course of Organic Reactions," Institute of Chemistry, 1932, p. 30-31); it also shows the absorption maximum at 2689Å* characteristic of the α-diketones; (*ii*) with hydrogen peroxide it yields *trans-cyclo*hexane-1:2-diacetic acid; (*iii*) with sodium amalgam, a mixture of 2:3-dihydroxy-*trans*-decalin and 2-keto-3-hydroxy-*trans*-decalin is obtained (*cf.* Lehmann and Kraetscheli, *Ber.*, 1934, **67**, 1867) and (*iv*) by boiling with sodium hydroxide it is converted into *trans*-hexahydrohydrindenol-β-carboxylic acid (III). This acid (III) has been obtained by Lehmann and Kraetschell (*Ber.*, 1935, **68**, 360) by boiling 2-keto-3-chloro-*trans*-decalin or 2-keto-3:3-dichloro-*trans*-decalin (*vide infra*) with alkali, the formation of the diketone (I) being postulated by them to be the intermediate in the reactions. The diketone (I) condenses with 4:5-diaminouracil and 4:5-diaminothiouracil to furnish in good yields 2′:3′-*trans*-decalolumazine (IV, R=O) and 2′:3′-*trans*-decalothiolumazine (IV, R=S) respectively.



(I)



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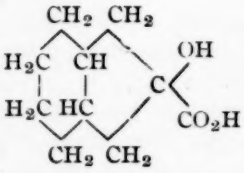

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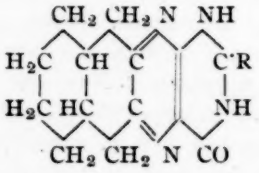


(IV)

It is now evident that the diketone, m. p. 99-100°, of Wallach and Wissenborn (*loc. cit.*) is 2:3-diketo-*trans*-decalin, probably formed by the contamination of their (*cis*) decalone with a small quantity of the *trans*-variety; the isomerisation of the *cis*-diketone (m. p. 88-89°) to the *trans* form (I) is not probable since the keto groups concerned in this compound are in the β-positions and the conditions of its formation are not likely to cause such an isomerisation (*cf.* Hückel *et al*, *Annalen*, 1927, **451**, 109; 1930, **502**, 99; Cook and Linstead, *J. Chem. Soc.*, 1934, 946).

* The author is indebted to Messrs C. T. Abichandani and S. K. K. Jatkar for these data.

Though during halogenations and condensation reactions only the carbon atom in position 3 of *trans*-β-decalone has been found to be reactive (Lehmann and Kraetschell, *loc. cit.*; Feu, McQuillon and Robinson, *J. Chem. Soc.*, 1937, 53; Cook and Lawrence, *ibid.*, p.877; Errington and Linstead, *ibid.*, 1938, 669), on oxidation with nitric acid *trans*-β-decalone yields as the main product *trans-cyclo*hexane-1:2-diacetic acid along with a small quantity of *trans-cyclo*hexane-1-carboxy-2-propionic acid (Kandiah, *J. Chem. Soc.*, 1931, 923). But in the above oxidation of *trans*-β-decalone with selenium dioxide, no 1:2-diketo-*trans*-decalin could he detected. In this connection, it should be of interest to note that Stiller and Rosenheim (*J. Chem. Soc.*, 1938, 353) by the oxidation of (3)-cholestanone with selenium dioxide could isolate only 2:3-diketocholestane.*

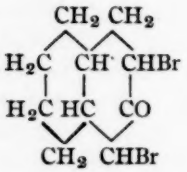
Rao and Kuppusamy (*loc. cit.*) brominated *trans*-β-decalone and obtained a monobromo compound which was considered by them to be 1- or 3-bromo-2-keto-*trans*-decalin.† As was pointed above, the substitutions in the case of either *cis*- or *trans*-β-decalone always take place at the carbon atom 3 so that we should expect this bromo compound to be 2-keto-3-bromo-*trans*-decalin. In attempting to verify this by the hydrolysis of the bromoketone to the corresponding known hydroxy compound, it was found that the bromine atom concerned was very inert to alkali and even the ketonic property of the compound was masked. Whereas the 3-chloro derivatives of both *cis*- and *trans*-β-decalones are readily hydrolysed by boiling with 1% sodium hydroxide solution (Lehmann and Kraetschell, *loc. cit.*; Cook and Lawrence, *loc. cit.*) and 2:3-dibromo-*trans*-decalin with 2% potash (Leroux, *Ann. chim. phys.*, 1910, *viii*, **21**, 496), the bromoketone was not hydrolysed even to an appreciable extent under these conditions. A trace of a product could be isolated which appeared to be identical with 2-keto-3-hydroxy-*trans*-decalin. It also remained unchanged by silver nitrate in boiling pyridine solution (*cf.* Dane, Wang and Schulte, *Z. physiol. Chem.*, 1936, **256**, 87; Heilbron, Jones and Spring, *J. Chem. Soc.*, 1937, 801; Heilbron, Jackson, Jones and Spring, *ibid.*, 1938, 102). On heating with potassium acetate and acetic acid in a sealed tube, the bromoketone did not yield the coresponding αβ-unsaturated ketone but a small amount of it appeared to isomerise to a compound (m.p. 122°) which on boiling with alkali changed back to the original bromoketone. Such an inertness of the bromine atom of this compound has, however, a

* We had just started this work as a natural sequel, when the paper of Stiller and Rosenheim appeared.

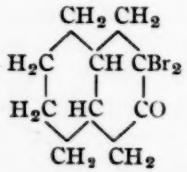
† The work was pursued in this direction because Rao and Kuppusamy have stated in their paper that neither of them will be able to continue the work.

parallel in the case of some of the bromoketones of the cholane group. Whereas the 4-bromo-(3)-ketones of the normal (*cis*-) series are easily debrominated in very good yields by boiling pyridine and also hydrolysed by methyl alcoholic potash, the 2-bromoketones of the *allo* (*trans*) series, *e.g.*, *allo*pregnandione, 3-keto-*bis*-nor*allo*cholanic acid, cholestanone, etc. are debrominated only with great difficulty in very poor yields (Butenandt and Mamoli, *Ber.*, 1935, **68**, 1850, 1854; Butenandt and Wolff, *ibid.*, p. 2091; Butenandt and Dannenberg, *ibid.*, 1936, **69**, 1158).

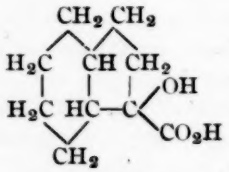
With two molecules of bromine, *trans*-β-decalone yields a dibromo compound (Rao and Kuppusamy, *loc. cit.*) which can be either 1:3-dibromo-2-keto-*trans*-decalin or 3: 3-dibromo-2-keto-*trans*-decalin (VI). An attempt is being made to decide between the two structures. The dibromo compound which is only very slowly acted on by shaking with 20% potash in the cold (*cf.* Wallach and Wissenborn, *loc. cit*), on boiling for a few hours yields a mixture from which *trans*-hexahydrohydrindenol-carboxylic acid (III) has been isolated. Since the dichloro-*trans*-β-decalone on boiling with alkali furnished the same acid (III) as the sole product (92% yield), Lehmann and Kraetshell (*loc. cit.*) have suggested that the latter should be represented as 3:3-dichloro-2-keto-*trans*-decalin.



(V)



(VI)



(VII)

Although 1:3-dichloro-2-keto-*trans*-decalin can also yield the acid (III), it is more probable that an acid of the structure (VII) should be formed from it. Therefore by analogy we represent the dibromo compound tentatively as (VI). It is interesting to note that Inhoffen (*Ber.*, 1937, **70**, 1695) definitely proved that the dibromo compound obtained by the bromination of 2-bromocholestanone (Butenandt and Schram, *Ber.*, 1936, **69**, 2779) or dibromination of cholestanone (Ruzicka, Bonhard, Fischer and Wirz, *Helv. Chim. Acta*, 1936, **19**, 1149) as the 2:4-dibromo and not 2:2-dibromocholestanone.

The 2:3-dihydroxydecalins are of great interest from the stereochemical point of view. Theoretically, a compound of such a structural formula can exist in three stereoisomeric forms, of which in the case of two the hydroxy groups occupy the *trans*- and in the third the *cis*- positions. The first two

should be resolvable whereas the third, being internally compensated, should not be resolvable. Leroux (*loc. cit*) has obtained from 2:3-dibromo-*trans*-decalin three isomeric dihydroxy compounds: (*i*) m.p. 160° (obtained by boiling the dibromo compound with potash), (*ii*) m. p. 141° and (*iii*) m.p. 125° (both by the hydrolysis of the acetates furnished by the action of silver acetate on the dibromo compound). The first (m. p. 160°) which appears to be identical with the one m. p. 168° of Lehmann and Kraetschell (*loc. cit.*), has been shown by Leroux (*loc. cit.*) to give rise to an oxide on dehydration so that the hydroxy groups in this compound might be expected to occupy the *cis*- position provided no change in configuration has occurred during dehydration. The *cis* configuration of this compound in a way seems to be supported by the fact that it is the highest melting of the isomers. The compound, m. p. 141°, now obtained by the reduction of 2:3-diketo-*trans*-decalin by sodium amalgam (a condition which generally favours the formation of *trans*- compounds), does not not also yield under the usual conditions the *iso*propylidene derivatives with acetone and hence appears to have the hydroxy groups in the *trans*-position (*cf.* Boeseken, *Rec. trav. chim.*, 1921, **40**, 566; Jack and Rule, *J. Chem. Soc.*, 1938, 188). The third compound, m. p. 125°, has been considered by Leroux to be a mixture of the other two products since on acetylation it yielded the acetates of the other two isomers. It has now been found that the reduction of 2:3-diketo-*trans*-decalin with aluminium amalgam yields a dihydroxy compound, m.p. 126°, obviously identical with the compound (m. p. 125°) of Leroux. The product is definitely homogeneous but less stable than the two other forms. During acetylation a change in its configuration occurs. In case the isomer, m. p. 168°, has the hydroxy groups in the *cis*- position, as we naturally expect, then this isomer, m. p. 126°, should have the hydroxy groups in the *trans*-position. Further work on the stereochemical configurations of these dihydroxydecalins is in progress.

EXPERIMENTAL.

2:3-*Diketo*-trans-*decalin*.—The following method was found to work better than that described by Rao and Kuppusamy (*loc. cit.*).

To *trans*-*β*-decalone (20 g.) dissolved in ethyl alcohol (50 c. c.) and water (15 c. c.) was added gradually with good shaking finely powdered selenium dioxide (15 g.). The separation of selenium was much accelerated by boiling on the steam-bath, and in half an hour the red turbid liquid became clear. After boiling for 3 hours, the clear brown solution was decanted off, the selenium washed with a little alcohol and from the solution the solvent was removed as far as possible first under ordinary and then under reduced

pressure. The residue was diluted with water, chilled, extracted with ether, the ethereal extract washed, dehydrated and the solvent removed. The residue on distillation passed between 95-135°/5 mm. (most of it passing steadily at 123-25°/5 mm.) and set to a crystalline mass, yield 14-16 g. After keeping for some hours, the crystalline mass (10-12 g.) was drained off from the liquid portion and the latter on careful repeated fractionation, gave decalone, b. p. 96-98°/5 mm. (2 g.) and a higher boiling liquid which slowly deposited the crystals. No other product could be isolated.

The solid crystalline product separated from dilute alcohol in beautiful glistening leaflets, m.p. 99-100°, or from water in shining prismatic needles, m.p. 100-101°. (Found: C, 72·18; H, 8·38. $C_{10}H_{14}O_2$ requires C, 72·29; H, 8·43 per cent). It is colourless and possesses a characteristic phenolic and camphor odour. In contrast to diosphenol it goes into solution in dilute alkali only very slowly. An alcoholic solution is coloured light yellow. It is soluble in all organic solvents and less in boiling and least in cold water With alcoholic ferric chloride it gives a red-violet colouration; it reduces Fehling's solution and alkaline permanganate.

Enolbenzoate of (II).—A solution of the diketone (0·5 g.) in pyridine (10 c. c.) was treated with benzoyl chloride (1 c. c.) drop by drop and after shaking well was set aside for about 18 hours. It was then diluted with water extracted with ether, the ethereal extract washed with sodium bicarbonate solution, water, dried and the solvent removed. The product obtained crystallises from petroleum ether in fine needles, m.p. 133°. (Found: C, 75·23; H, 6·46. $C_{17}H_{18}O_3$ requires C, 75·53; H, 6·70 per cent). It gives no colouration with ferric chloride.

Disemicarbazone.—When the diketone (0·8 g.) in methyl alcohol (10 c.c.) was mixed with an aqueous solution of semicarbazide hydrochloride (8·5 g.) and sodium acetate (1·5 g.), the semicarbazone began to separate. From hot dilute acetic acid it separated as a microcrystalline powder, m.p. 264-65°. (Found: N, 29·92. $C_{12}H_{20}O_2N_6$ requires N, 30·0 per cent). Rao and Kuppusamy (*loc. cit.*) record m.p. 257°. It is sparingly soluble in alcohol.

Dioxime.—To the diketone (0·8 g.) dissolved in little methyl alcohol was added hydroxylamine hydrochloride (0·8 g.), sodium hydroxide (2*N*, 15 c. c.) and the solution refluxed on the steam-bath for 20 minntes, cooled, diluted, and acidified whereby the dioxime was thrown down. It crystallised from alcohol in shining rhombic plates, m.p. 229° (decomp.). (Found: N, 14·35. $C_{10}H_{16}O_2N_6$ requires N, 14·29 per cent). An alcoholic solution of the dioxime gave with a solution of nickel sulphate, the rose-red precipitate of the nickel salt characteristic of the dioximes of the α-diketones.

The oximation by the method of Forster and Dunn (*J. Chem. Soc.*, 1909, **95**, 431) gave the same product and no isomeric forms could be obtained.

The reduction of the dioxime by sodium and alcohol furnished in poor yield a product which does not appear to be the corresponding diamine; however, aluminium amalgam in moist ether solution gave a product which appears to be an amine. This is being further investigated.

Quinoxaline.—The diketone (0·4 g.) and *o*-phenylenediamine (0·25 g.) in glacial acetic acid (8 c. c.) was refluxed for 2 hours, the brown-red solution cooled and diluted with water whereby the quinoxaline was precipitated (0·5 g.). This crystallised from water (charcoal) in glistening rhombic plates, m.p. 177-78°. (Found: N, 11·68. $C_{16}H_{18}N_2$ requires N, 11·77 per cent).

2′:3′-trans-*Decalolumazine* (IV, R=O).—The diketone (0·8 g.) and 4:5-diaminouracil sulphate (1 g.) in glacial acetic acid (50 c. c.) and water (100 c. c.) were refluxed for 45 minutes during which the colourless solution became gradually yellow. On allowing to stand overnight the condensation product separated in fine prismatic needles, yield 0·8 g.; little more being obtainabie from the mother-liquors on concentration. From dilute acetic acid it separated in clusters of fine colourless needles, m. p. about 315°. (Found: N, 20·32. $C_{14}H_{16}O_2N_4$ requires N, 20·44 per cent). It is moderately soluble in alcohol and goes gradually into solution in alkali, the solution exhibiting a blue fluorescence in a quartz lamp.

2′:3′-trans-*Decalothiolumazine* (IV, R=S).—A mixture of the diketone (0·8 g.), 4:5-diaminothiouracil (1 g.), water (100 c. c.) and glacial acetic acid (59 c. c.) were refluxed for 45 minutes, and on cooling a crystalline mass (0·9 g.) separated. Recrystallising from dilute acetic acid, it was obtained as light yellow needles, m.p. 295°. (Found: N, 18·89. $C_{14}H_{16}ON_4S$ requires N, 19·44 per cent). It readily dissolves in aqueous sodium hydroxide and the solution appears to show a very feeble green flourescence. It is desulphurised by yellow oxide of mercury.

Oxidation of the Diketone by Hydrogen Peroxide.—To the diketone (0·5 g.), dissolved in methyl alcohol (10 c.c.) and sodium hydroxide (5 c.c.; 2*N*) was added gradually with shaking a solution of hydrogen peroxide (5 c.c. of 30% diluted to 20 c.c.). The solution which warmed up was left aside overnight. On acidifying with hydrochloric acid, a crystalline product separated in nearly quantitative yield. Recrystallised from dilute alcohol, it had m.p. (and mixed m.p. with a sample of *trans-cyclo*hexane-1:2-diacetic acid) 167°. [Found: Equiv., 100·3. $C_{10}H_{16}O_4$ (dibasic) requires Equiv., 100].

Reduction of Diketo-trans-*decalin*.—(*i*) A solution of the diketone (1 g.) in methyl alcohol (150 c.c.) and water (20 c.c.) was treated with sodium amalgam (4% ; 30 g.) and stirred well for 2 to 3 hours. The solution was then filtered, the light yellow filtrate acidified with hydrochloric acid and the alcohol removed as far as possible under reduced pressure. The residue was diluted with a little water, extracted thoroughly with ether and from the ethereal extract a solid was obtained (0·6 g.) which on fractional crystallisation from water yielded (*i*) a product in shining rhombic plates, m.p. 141°, identified to be 2 : 3-dihydroxy-*trans*-decalin (Found: C, 70·28 ; H, 10·32. $C_{10}H_{18}O_2$ requires C, 70·59 ; H, 10·59 per cent). and (*ii*) another product crystallising in needles, m. p. 134°, identified to be 2-keto-3-hydroxy-*trans*-decalin (Found : C, 71·18 ; H, 9·31. $C_{10}H_{16}O_2$ requires C, 71·43 ; H, 9·53 per cent). A small amount of tarry matter was also obtained.

The dihydroxy compound, m.p. 141°, was unchanged by standing for 24 hours with dry acetone and little sulphuric acid.

(*ii*) To the diketone (1 g.) dissolved in ether (200 c.c.), aluminium amalgam (1·5 g.), prepared according to Vogel, was added. Water was added to it from time to time to keep the reaction going on for about 24 hours. Then the ether was separated from the sludge, the latter extracted with ether and the combiued ether extract washed, dried and on evaporation of the solvent furnished a product which crystallised from dilute alcohol in shining plates and from petrol (70-90°) in clusters of fine needles, m.p. 126°. (Found : C, 70·20 ; H, 10·29. $C_{10}H_{18}O_2$ requires C, 70·59 ; H, 10·59 per cent). This is definitely homogeneous and is evidently an isomeric 2 : 3-dihydroxy-*trans*-decalin.

trans-*Hexahydrohydrindenol carboxylic Acid*.—To obtain the corresponding *cis*-isomer, Wallach and Wissenborn (*loc. cit.*) heated the diketone with potash (1 : 2) in an autoclave at 120-30° for 30 minutes. Under the following conditions a quantitative yield can be obtained.

The diketone (0·5 g.) was refluxed for 3 hours with *N*-sodium hydroxide (10 c.c.), the alkaline solution thoroughly extracted with ether to remove any unchanged material, filtered and acidified whereby a solid was precipitated which was filtered and crystallised from boiling water, m.p. 133-34°. [Found : Equiv., 184·5. $C_{10}H_{16}O_3$ (monobasic) requires Equiv., 184·1].

2-*Keto*-3-*bromo*-trans-*decalin*.—This compound was prepared essentially according to the method of Rao and Kuppusamy (*loc. cit.*), modifications neither improving the yield nor giving immediately a crystalline product. It crystallised from alcohol, m.p. 150°. (Found : Br, 32·0. $C_{10}H_{15}OBr$ requires Br, 32·35 per cent). The residue does not appear to be homo-

geneous and no other crystalline product could so far be isolated. It did not give an oxime by the pyridine method.

On boiling the bromoketone with 10% potash for 6 hours or 35% soda solution for 2-4 hours or methyl alcoholic potash for a number of hours, no appreciable change was noticed, most of the material being recovered unchanged. By working up the mother-liquors of a number of experiments, however, a small quantity of a product, m.p. 130° (too small for further crystallisation) could be isolated which appears to be 2-keto-3-hydroxy-*trans*-decalin, since it caused no appreciable depression of the m.p. of an authentic specimen.

With silver benzoate the substance yielded a product, m.p. 122°, which on boiling with potash for a number of hours gave back the original bromo compound. On heating the bromo compound with potassium acetate and acetic acid in a sealed tube at 170-80° for 5 hours, most of the product was recovered unchanged and a small quantity of a compound, m.p. 122°, was produced. These reactions are being further studied.

Dibromo-trans-*β-decalone*.—The dibromo compound was obtained by the method of Rao and Kuppusamy. As the temperature of bromination is increased, the product loses the tendency to crystallise and probably isomers are also produced. The dibromo compound is only very slowly affected by 20% potash in the cold but on boiling for 3-4 hours, *trans*-hexahydrindenol carboxylic acid (m.p. and mixed m.p. with authentic sample, 134°), could be isolated from the alkaline mother-liquors. The yield of this acid, however, is low.

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POTENTIOMETRIC STUDIES IN DIAZOTISATION. DETERMINATION OF AROMATIC AMINES.

By Balwant Singh and G Ahmed.

A number of amines in presence of hydrochloric acid were titrated potentiometrically (pt-electrode calomel electrode in amine) against a sodium nitrite solution. E. M F. was found to rise steadily with the addition of standard sodium nitrite till the equivalence point At the equivalence point there was a sharp jump in potential in each case.

Aromatic amines are quantitatively converted into diazo-compounds by nitrous acid, *i.e.*, by nitrite in acid medium. The end-point of the diazotisation is reached with the first slight excess of nitrous acid that is stable for some time, and may be detected with potassium iodide-starch as indicator. This indicator is by no means to be added directly to the reaction mixture because it responds instantaneously to nitrous acid and, since the diazotisation requires a certain time, it would, change much too soon The course of the reaction is followed by spotting tests on potassium iodide-starch paper. The reaction is complete when on stirring after each addition, and testing with the iodide-starch paper, a distinct blue colour is obtained at once. A drop of the solution is removed on a glass rod at intervals and placed on the iodide-starch paper. Whenever the blue colour is obtained, and this colour persists when another test is made after a few minutes, the reaction is complete. To obviate the difficulty of finding the end-point by this visual method, the titrations were conducted potentiometrically.

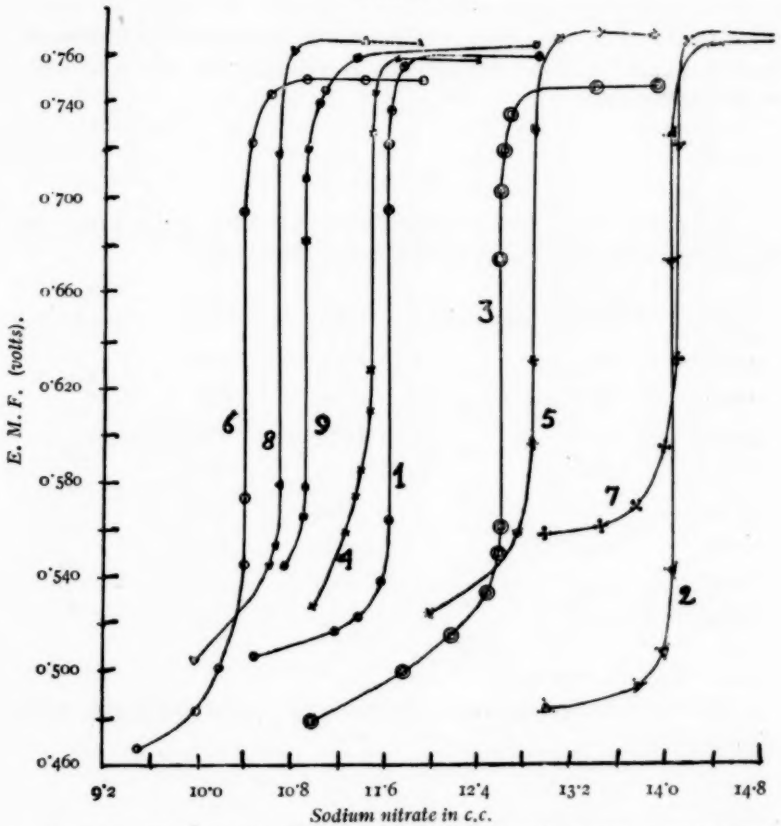
When the solution of an aromatic amine is diazotised free nitrous acid is formed at the completion of the reaction. A solution of nitrous acid in contact with a platinum electrode, gives a definite potential. E. Muller and E Dachselt (*Z. Elektrochem.*, 1925, **31**, 662) studied the titration of aniline, xylidine, toluidine and amino-azobenzene disulphonic acid. They found that there is a jump in potential at the equivalence-point of the titration of the acid solution of the aromatic amine with sodium nitrite. The potential requires some time to become steady during the titration. Attempts were made to accelerate the reaction by using higher temperatures. It was found that side-reactions occur, causing errors; 3 % at 60°; 0·5% at 30°. At 20° or below the results are very accurate. It is not necessary, therefore, to cool the mixture in ice during the titration.

A systematic study of the application of the potentiometric method to diazotisation has been taken up during the course of this investigation.

Experimental.

A bright platinum electrode immersed in a solution of the amine to be titrated, was coupled with a saturated calomel electrode. The cell was placed in a water bath, the temperature of which was maintained at 10°. The E.M.F. of the cell was read on a potentiometer scale.

Fig. 1



Curves 1-9 refer respectively to *o*-, *m*-, and *p*- nitroanilines, *o*- and *p*- aminobenzoic acids, sulphanilic acid, *p*-, chloroaniline, 3-nitro-4-ami nophenetole and 2-amino-4-nitrophenol.

o-Nitroaniline (m.p. 70°), *m*-nitroaniline (m.p. 114·2°), *p*-nitroaniline (m.p. 146·5°), *o*-aminobenzoic acid (m.p. 144·8°), *p*-aminobenzoic acid (m.p. 187·0°), sulphanilic acid (m.p. 172·5°), *p*-chloroaniline (m.p. 70·5°), 3-nitro-4-aminophenetole (m.p. 112·0°) and 2-amino-4-nitro-

phenol (m.p. 142·9°) were quantitatively determined by titrating them against a standard solution of sodium nitrite in the presence of hydrochloric acid by the potentiometric method. On each addition of the titrant, the mixture was thoroughly stirred by means of a mechanical stirrer. The potentiometric readings were recorded two to five minutes after the addition of the reagent when the potential acquired a steady value. For an accurate determination a micro-burette was used to add the standard solution near the equivalence-point.

A series of potentiometric titrations were performed with different amounts of each substance. One titration, as typical of that set, is recorded in the following table.

TABLE I.

Titration of 0·3231 g. of *o*-nitroaniline mixed with 20 c.c. water and 25 c.c. conc. hydrochloric acid, against sodium nitrite (M/5).

$NaNO_2$.	E.M.F. (volts)	*E/C.* (m. volts/c.c.)	$NaNO_2$.	E.M.F. (volts)	*E/C.* (m. volts/c.c.)
11·200 c.c.	0·515		11·700 c.c.	0·694	
		30			1080
11·400	0·521		11·725	0·721	
		50			600
11·500	0·526		11·750	0·736	
		80			360
11·550	0·530		11·775	0·745	
		120			200
11·600	0·536		11·800	0·750	
		200			120
11·625	0·541		11·825	0·753	
		320			80
11·650	0·549		11·850	0·755	
		520			40
11·675	0·562		11·900	0·757	
		5280 (maximum)			

The titrations, one for each substance, are represented by the curves given in Fig. I.

DISCUSSION.

In these titrations, with the addition of the standard sodium nitrite, the E.M.F. rose steadily till the equivalence-point. At the equivalence-point there was a sharp jump in potential in each case. For the addition of 0·025 c.c. of the reagent the value of the inflection-potential was of the order of about 90 to 136 millivolts for the nitroanilines, 86 to 110 millivolts for the

aminobenzoic acids, 220 to 136 milllvolts for sulphanilic acid, 80 to 90 millivolts for *p*-chloroaniline, 130 to 140 millivolts for 3-nitroaminophenetole and 100 to 120 millivolts for 2-amino-4-nitrophenol.

After the equivalence-point there was again rise in the potential but with further addition of the reagent the potential assumed a constant value, followed by a steady fall. From the volume of the sodium nitrite required in each titration, corresponding to the equivalence-point, the amount of the amine was calculated. The values obtained are compared in the following table with the amount of the amine taken.

TABLE II.

	Substance.	Amount (taken) gms.	Amount (found) gms.
1.	*o*-Nitroaniline	0·3231	0·3227
		0·4260	0·4255
		0·5250	0·5246
		0·6894	0·6899
		0·8434	0·8428
		1·1016	1·1014
2.	*m*-Nitroaniline	0·3900	0·3895
		0·4504	0·4502
		0·6019	0·6022
		0·7212	0·7217
3.	*p*-Nitroaniline	0·3490	0·3488
		0·4858	0·4854
		0·5642	0·5640
		0·6518	0·6517
4.	*o*-Aminobenzoic acid	0·3173	0·3168
		0·4748	0·4744
		0·5044	0·5039
		0·6390	0·6388
5.	*p*-Aminobenzoic acid	0·3552	0·3547
		0·3848	0·3846
		0·4864	0·4864
		0·5652	0·5653
		0·6572	0·6567

TABEE II (*cotnd.*).

	Substance.	Amount (taken) gms.	(found) gms.
6.	Sulphanilic acid	0·1812	0·1810
		0·4046	0·4043
		0·5752	0·5752
		0·7654	0·7649
7	*p*-Chloraniline	0·3608	0·3607
		0·4212	0·4209
		0·5240	0·5237
		0·7620	0·7617
8.	3-Nitro-4-aminophenetole	0·3924	0·3922
		0·4118	0·4112
		0·5023	0·5021
		0·7010	0·7004
		0·8012	0·8007
9.	2-Amino-4-nitrophenol	0·3382	0·3380
		0·5552	0·5549
		0·6530	0·6527
		0·7382	0·7380

From the above results it is evident that the amines can be determined quantitatively by the potentiometric method, by titrating them against a standard solution of sodium nitrite.

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RELATIONS BETWEEN CHEMICAL ACTIVITY AND ABSORPTION IN THE ULTRAVIOLET OF CERTAIN ORGANIC MOLECULES. PART I. STUDY OF THE ABSORPTION SPECTRA OF THE CHLORO DERIVATIVES OF THE SUBSTITUTED AMIDES OF MALONIC ACID.

By Mme. Ramart, K. G. Naik and C. M. Mehta.

In the present study it is expected to throw some light on the relationship between the chemical activity of the chloro derivatives of the various substituted amides of malonic acid of the type: $Cl_2 : C : (CONHR)_2$, $Cl_2 : C : (CONH_2).(CONHR)$ and $(Cl)(ClCH_2) : C : (CONHR)_2$, where R is phenyl, tolyl, etc.

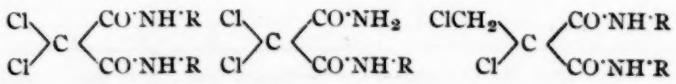
The results of absorption spectra of the above compounds have been compared with the absorption spectra measurements of the compounds of the type $H_2 : C : (CONHR)_2$ studied by Ramart, Naik and Trivedi (*Bull. Soc. Chim.*, 1934, **1**, 525).

The comparison of these two corresponding sets of curves shows that the transformation of methylene group CH_2 into CCl_2 group produces a distinct change in the nature of the curves.

In 1913 Victor Henri proposed a law stating that 'Along with several other effects, substances of which the molecules are reactive, also show a strong exaltation in their power of absorbing ultraviolet rays.' (*Compt. rend.*, 1913, **156**, 1997).

More recently one of us (Ramart, *Compt. rend.*, 1932, **194**, 7216) announced that, as long as the internal condition of a chromophore remains the same, the chemical activity of this chromophore with regard to another molecule (with which it may enter into reaction) remains unaltered. Numerous examples of this excellent relationship between the chemical activity and the absorption in the ultraviolet of organic molecules exist in literature.

Herein are embodied the investigations with regard to the relationship between the chemical activity of the chloro derivatives of the various substituted amides of malonic acid of the types :

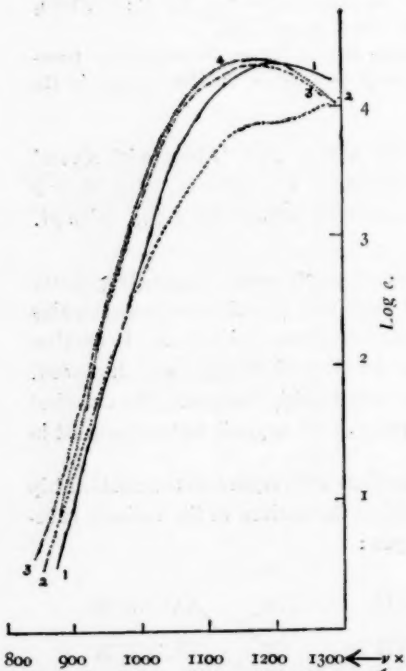


and their absorption in the ultraviolet. The following substances were obtained in optically pure condition for spectrographic examination.

(1) Dichloromalon-di-phenylamide, (2) dichloromalon-di-*o*-tolylamide, (3) dichloromalon-di-*p*-tolylamide, (4) dichloromalon-di-*m*-(chloro)-tolylamide, (5) dichloromalon-di-1:3:4-xylidide, (6) dichloromalon-di-heptylamide, (7) dichloromalon-dibenzylamide, (8) dichloromalon-mono-*p*-tolylamide, (9) dichloromalon-mono (chloro)-phenylamide, (10) chloromethyl-chloromalon-diphenylamide, (11) chloromethyl-chloromalon-di-*o*-tolylamide, (12) chloromethyl-chioromalon-di-*p*-tolylamide.

The relationship which exists between the chemical activity of the simple amides of malonic acid and their absorption in the ultraviolet has been already studied (*cf.* Ramart, Naik and Trivedi, *Bull. Soc. Chim.*, 1934, **1**, 525).

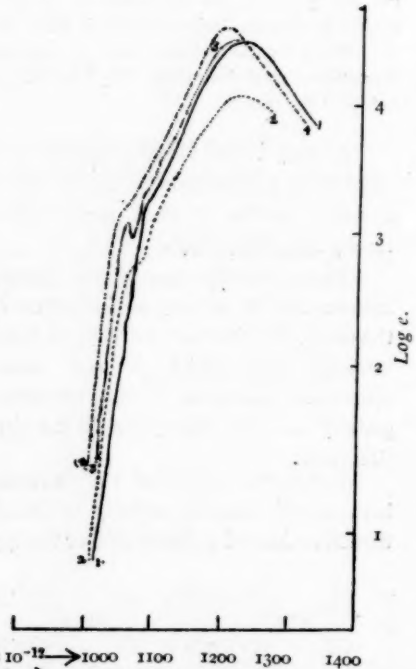
FIG. 1.



800 900 1000 1100 1200 1300 ← $\nu \times 10^{-12}$ → 1000 1100 1200 1300 1400
3750 3333 3000 2750 2500 2307 ← λ → 3000 2730 2500 2307 2142

Curves 1—4 refer respectively to dichloromalon diphenylamide,- and -di-*o*-, *p*- and *m*-tolylamides.

FIG. 2.

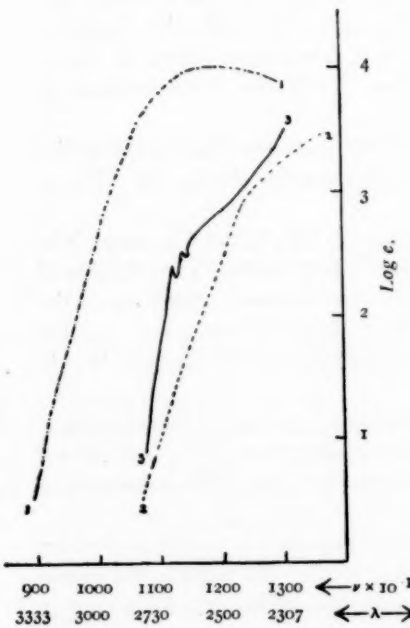


Curves 1—4 refer respectively to malon-diphenylamide and -di-*o*-, *m*-and *p* tolylamides.

EXPERIMETAL.

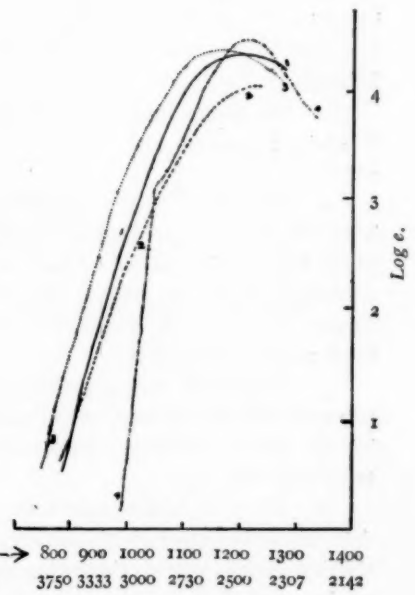
The examination was made by means of the Chemists' spectrograph of Zeiss. The source of ultraviolet rays was Challonge hydrogen lamp. It was supplied by the electric current from a 2,000 volt sector (transformed from 110 volts). The solvent and the solution of the substance to be studied were placed in two similar tubes with end quartz plates. The tube containing the solvent is preceded by sector photometer which reduces the flux of light by means of an adjustible angle of overture. The system of two tubes is followed by a Hüffner prism which brings the two juxtaposed spectra on the sensitised photographic plates. The pairs of spectra thus produced can be photographed between wave-length scales marked in ordinary Angstrom wave-length units.

FIG. 3.



Curves 1-3 refer respectively to dichloro-malon-monochlorophenylamide,- diheptylamide and,- dibezylamide.

FIG. 4.



Curves 1-4 refer respectively to chloro-malon-diphenylamide, -di- *o*, di-*p*-tolylamide and malon-di-*p*-tolylamide.

The rssults of spectrographic examinatious are represented by marking the position of the absorption bands (as shown with reference to the wavelength scale) along the abscissae, and the corresponding molecular extinctions are marked along ordinates.

The molecular extinction is obtained from the following formula:—

$$E=\frac{1}{cd}\ \log\ 10\frac{I_o}{I}$$

where, I_o is the intensity of the incident ray; I, the intensity of the emerging ray; E, the coefficient of molecular extinction, and c, the molecular concentration of the solution under experiment; d, the length of the column of the solution through which the ray is passing.

The above formula could also be expressed as

$$E=\frac{1}{cd}\ \log\ 10\frac{2\pi}{\alpha}$$

where, α is the angle of the sector, through which the light is passing. Sometimes instead of the coefficient of the molecular extinction itself, its logarithm is plotted along the ordinates. The curves given in Fig. 1 represent the results of our observations, which can be summarised as follows:

1. The general nature and sense of the corresponding curves of the simple malonamides and the chloro derivatives are similar (*cf.* Figs. 1 and 2).

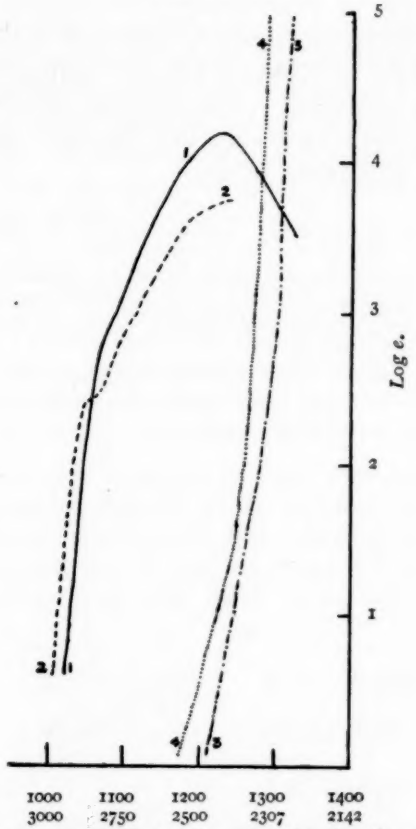
2. The effect of the transformation of CH_2 into CCl_2 group, is to bring about enhanced general absorption. This results in the shifting of curves towards the visible region. Again, the secondary band due to the methylene group disappears in the curves of the chloro derivatives. (*cf.* the curves of malon-diphenylamide and dichloromalon-diphenylamide in Figs. 2 and 1 respectively).

3. The nature of the redicals attached to the carbonyl groups determines the position and nature of the curves of absorption. This is illustrated by the curves of dichloromalon-diphenylamide and dichloromalon-di-*o*-tolylamide (Fig. 1).

4. The molecular weight of the radicals attached to the imino grouping determines the extent of absorption. The heavier the radicals, the greater shifting of the curve of absorption towards the visible (*cf.* Figs. 1 and 3, as also 1 and 5).

5. When the radicals have the same number of carbon atoms, absorption is determined by the constitution of such radicals. Open-chain radicals give simple curves; whereas ring structures give many banded curves,

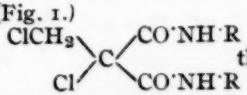
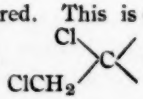
FIG. 5.



Curves 1-4 refer respectively to malon-mono-phenylamide, -*o*-tolylamide, malon-diheptylamide and malonamide

This is illustrated by the cases of dichloromalon-diheptylamide and dichloromalon-dibenzylamide. (curves 2 and 3 in Fig. 2.)

6. In the case of the three tolylamide derivatives, the position of the absorption curves is affected by the proximity or distance of the methyl group to or from the amino grouping (Fig. 1.)

7. In the case of the derivatives  the secondary band due to the methylene group situated between the the two carbonyl groups has disappeared. This is due to the fact that the CH_2 group has been converted into  (Fig. 4.)

THE COLLEGE, BARODA.

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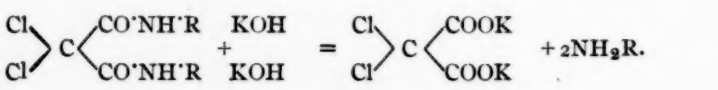
RELATIONS BETWEEN CHEMICAL ACTIVITY AND ABSORPTION IN THE ULTRAVIOLET OF CERTAIN ORGANIC MOLECULES. PART II. VELOCITY OF SAPONIFICATION OF THE CHLORO DERIVATIVES OF THE SUBSTITUTED AMIDES OF MALONIC ACID.

By K. G. Naik, R. K. Trivedi and C. M. Mehta.

In order to investigate chemical activity of the chloro compounds as expressed by their velocity of saponification, a number of various substituted amides of malonic acid were made to react with standard alcoholic potash solution and titrated with standard hydrochloric acid at regular intervals.

The velocity of saponification depends upon the nature of the radical attached to the saponifiable imino(NH) grouping. It also depends upon the position of the methyl group with regard to the saponifiable imino group.

The investigations with regard to the above were undertaken with a view to correlate the chemical activity of these substances as expressed by the velocity of soponification with absorption in the ultraviolet. The velocity of saponification was studied with the help of a standard solution of caustic potash. The reaction which takes place can be represented as follows :—



(where R = phenyl, tolyl, etc.).

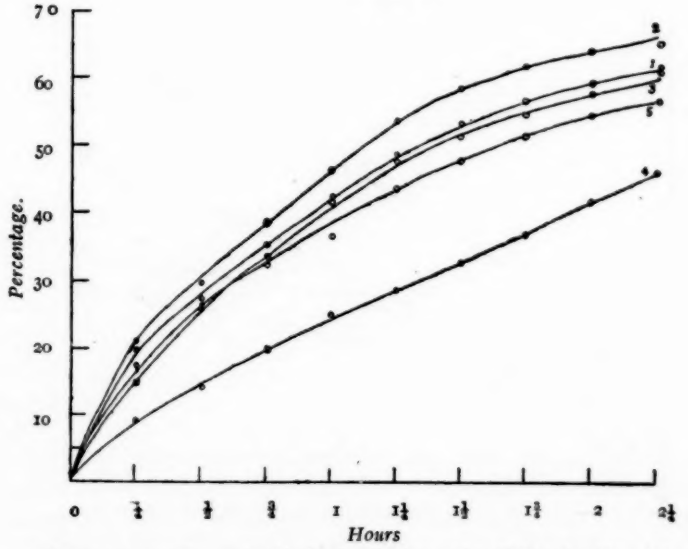
A weighed quantity of the chloro derivative was added to 100 c. c. of $N/1$-alcoholic caustic potash solution so as to make the concentration of the solution 0·05 molecules per litre. Ten ampoules, each containing 5 c c. of the above reacting mixture, were put in an electrically regulated thermostat maintained at 30°, it being previously ascertained that the chlorine atoms in the compounds studied remained totally unaffected even up to 40°. (C. B., **B**, **25**, 2994). At the end of each quarter of an hour, one of the ampoules was withdrawn and the contents titrated with a standard solution of hydrochloric acid (0·125N).

In Fig. 1 are traced the curves showing the velocity of saponification of (1) dichloromalon-diphenylamide, (2) dichloromalon-di-*p*-tolylamide, (3) dichloromalon-di-*m*-tolylamide, (4) dichloromalon-di-*o*-tolylamide and (4) dichloromalon-di-1 : 3 : 4-xylidide. It will be seen from these curves that

the curve representing the velocity of saponification of compound (1) lies between those of compounds (2) and (3) ; and again the curve for the velocity of saponification in the case of compound (4) is situated much below the curves for 1, 2, and 3. It may be pointed out here that the general position and sense of the curves 1, 2, 3 and 4 is similar to those of their respective curves of their absorption spectra, (*cf.* Fig. 1 in each of the parts I and II). The curve in the case of compound (5) has got a peculiar position which can be ascribed to the presence of two methyl groups in the xylidide radical

Fig. 2 shows the curves of the velocity of saponification of (1) dichloromalon-di-heptylamide, (2) dichloromalon-di-propylamide, (3) dichloromalon *mono-p*-tolylamide, (4) dichloromalon-*mono*chlorophenylamide. A study of these curves will immediately reveal that the velocity is enhanced in the case of (1) where there is a heavy heptyl radical with an open-chain. In the case of (2) the velocity of sapcnification is reduced. This may be attributed to the nature of the comparatively light propyl radical. During

FIG. 1.



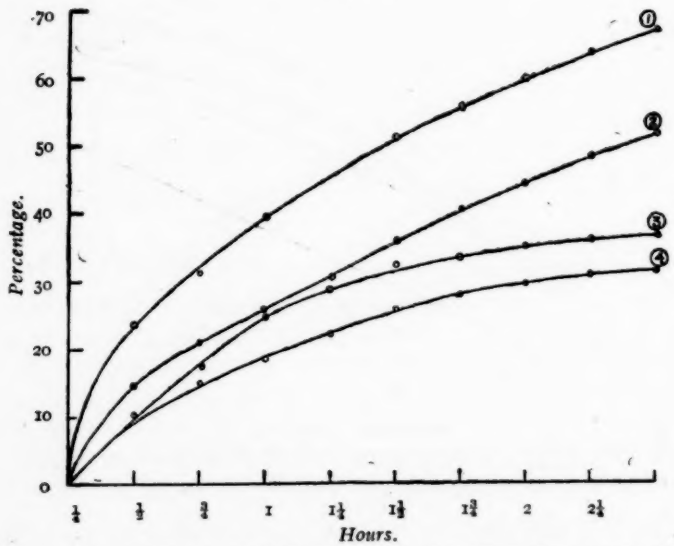
Curves 1—5 refer respectively to dichloromalon-diphenylamide, -di-*p*- tolylamide, -di-*m*-tolylamide dichloride, -di-*o*-tolylamide and di- 1 : 3 : 4-xylidide.

a comprehensive study of the velocity of esterification as well as on the saponification of esters, Kellas has shown that the molecular weight as well

as the molecular volume of the radicals attached to such a reactive group, appear to be an important factor in the determination of the velocity of reaction (*Z. physikal. Chem.*, 1897, **24**, 221). With regard to the curves 3 and 4 in Fig. 2, it will be seen that although their nature is similar to those of curves 2 and 1 respectively in Figs. 1, the velocity of saponification as shown by the former curves is different, on account of the presence of only one tolyl or phenyl radical in the case of 3 and 4 in place of two such radicals as in the case of 2 and 1.

In Fig. 3 are traced the curves for the velocity of saponification of the three derivatives of methyl malonic acid *viz.* (1) chloromethyl-chloromalon-diphenylamide, (2) chloromethyl-chloromalon-di-*p*-tolylamide and (3) chloromethyl-chloromalon-di-*o*-tolylamide. The velocity of saponification is reduced in all these cases as compared with that of the corresponding derivatives of malonic acid (*vide* Figs. 1, 2 and 4). This difference is in excellent agreement with the difference which exists between the curves of absorption spectra of these two classes of derivatives. (*vide* Figs. 1 and 4 of Part I).

FIG. 2.

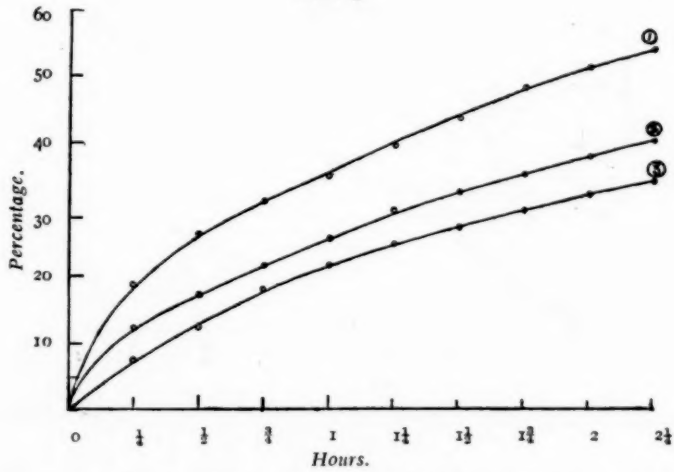


Curves 1-4 refer respectively to dichloromalon-diheptyl-, -dipropyl-, -mono-*p*-tolyl- and -monochlorophenyl-amides.

The results of the measurements of the velocity of saponification are summarised in the accompanying (Table)

With regard to the saponification of all the substances mentioned in Table I. the results give merely the sum total of the velocity of reaction of two saponifiable groups. When we consider the mechanism of the saponification it may be apparent that the velocity of saponification will not be the same for each group in the molecule. It can easily be conceived that the sopanifiable radical being fixed on the same carbon atom will have mutual influence on the reactivity of each other. Consequently, the rate of saponification must slowly change as one of the groups is gradually being saponified. This will be evident also from the nature of the curve which ascends steeply in the beginning.

FIG. 3.



Curves 1—3 refer respectively to chloromethyl-chloromalon-diphenyl-, -*p*-tolyl-, and -*o*-tolylamide

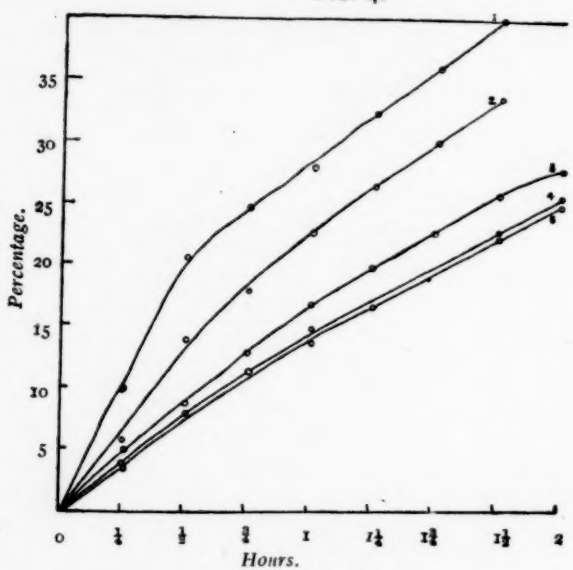
From Table I it can be seen that when the nitrogen carries aliphatic radicals, the velocity of saponification is greater than when it carries an aromatic radical having the same number of carbon atoms. (*vide* curve 1 in Fig. 2 and curves 2, 3, 4 in Fig. 1).

When we compare the velocity of saponification of the chloro derivatives of the malon-tolylamides the augmentation in the velocity of saponification could be represented as follows :—

ortho →	*meta* →	*para*.
(lowest)	(intermediate)	(highest).

This difference in the velocity of saponification could be attributed to the position of the methyl group with regard to the saponifiable imino grouping. When the methyl group is very near the imino group, as in the case of *ortho* derivative, the velocity is considerably low ; but it is enhanced as the position of the group is receding away from the saponifiable -NH- grouping.

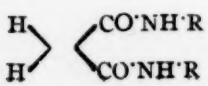
FIG. 4.



Curves 1—5 refer respectively to malon-diheptyl-, diphenyl- and -di-*o*-, -*m*-, *p*-tolylamides.

In Part I it was stated that the more active the molecule, the higher is its absorption in the ultraviolet and that the absoption bands are shifted more towards the visible. The above fact appears to be substantiated by this comparative study of the corresponding curves for velocity of saponification on the one hand and for the absorption in the ultraviolet on the other.

Ramart, Naik and Trivedi (*Bull. Soc. chim.*, 1934, **1**, 525) studied the velocity of saponification of the amides of the type,



where R is phenyl, tolyl, etc.

TABLE I.

Names of compounds.	*h. m.* 0·15	*h. m.* 0·30	*h. m.* 0·45	*h. m.* 1·0	*h. m.* 1·15	*h. m.* 1·30	*h. m.* 1·45	*h. m.* 2·0	*h. m.* 2·15
Dichloromalon-di-phenylamide.	20·00	27·50	35·50	42·50	48·75	53·12	56·25	59·25	61·50
Dichloromalon-di-*p*-tolylamide.	21·25	30·00	38·75	46·25	53·75	58·25	61·75	63·75	65·00
Dichloromalon-di-*m*-tolylamide-dichloride.	15·00	25·75	33·75	41·75	47·50	51·25	54·50	57·50	60·75
Dichloromalon-di-*o*-tolylamide.	9·25	11·25	20·00	25·00	28·75	32·50	36·75	41·25	45·75
Dichloromalon-di-1 :3 : 4-xylidide.	17·50	26·25	32·50	36·88	43·75	47·75	51·25	54·37	56·25
Dichloromalon-di-heptylamide.	23·75	31·25	39·40	45·62	51·25	55·50	60·00	63·75	67·50
Dichloromalon-di-propylamide.	15·00	21·25	26·25	30·75	36·00	40·75	45·00	48·75	51·85
Dichloromalon-mono-*p*-tolylamide.	10·00	17·50	25·75	29·25	32·50	33·50	35·50	36·25	36·75
Dichloromalon-mono-chlorophenylamide.	10·00	15·00	18·75	22·50	26·25	28·25	30·00	31·15	31·75
Chloromethyl-chloromalon-di-phenylamide.	12·50	17·50	21·87	25·75	30·00	32·50	35·00	38·00	40·62
Chloromethyl-chloromalon-di-*p*-tolylamide.	18·75	26·25	31·25	35·00	39·25	43·75	48·00	51·25	53·75
Chloromethyl-chloromalon-di-*o*-tolylamide.	7·50	12·50	18·10	21·75	25·00	27·50	30·00	32·50	34·50

% Saponified during the time shown in the column at the top of the Table.

In Fig. 4 are shown the curves for the saponification of (1) malon-di-heptylamide, (2) malon-di-phenylamide. (3) malon-di-*o*-tolylamide, (4) malon-di-*m*-tolylamide and (5) malon-di-*p*-tolylamide In the first place the remarkable difference between the velocity of saponification of the amides of malonic acid on one hand and that of the chloro-derivatives on the other (*vide* Figs. 1), may be attributed to the transformation of the methylene group CH_2 into CCl_2 group of the substituted amides. Such a transformation must profoundly change the nature of the molecule. When the molecule undergoes a change, the initial condition is altered. Consequently the chemical activity of the imino group may also be altered.

They found that in the case of three malon di-tolylamides, the augmentation of the velocity of saponification proceeds as *para*→ *meta*→*ortho*, which is in exact opposition to the velocity of saponification of their dichloro derivatives, for in the latter case it proceeds as *ortho*→*meta*→*para*. This apparent anomaly could be explained by the fact that the amides are capable of existing in a condition of tautomeric equilibrium in which the dichloro derivatives cannot. When in the case of chloro derivatives such dynamic isomerism is not easily possible, the velocity may be solely influenced by the position of the methyl group with regard to the saponifiable imino group as has already been mentioned.

Finally the results of the observations discussed above may be presented as follows:—

(1) The velocity of saponification of chloro derivatives is in full accord with their absorption spectra.

(2) The velocity of saponification depends upon the nature of the radical attached to the saponifiable imino grouping.

(3) In the case of chloro derivatives of malon-di-tolylamides, the velocity of saponification depends upon the position of the methyl group with regard to the saponifiable imino grouping.

(4) The transformation of methylene CH_2 group into CCl_2 group, enhances the absorption in the ultraviolet as also the chemical activity of the molecules, as expressed by the velocity of saponification, which is in accordance with the observation of Ramart, Naik and Trivedi (*loc. cit.*).

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COMPOSITION OF BOILED OIL.

By S. Chatterjee, A. Saha and M. Goswami.

Boiled oil has been shown to have Diene value indicating the presence of conjugated double bond. The development of the conjugated state has been connected with the presence of metals in the finely divided condition. The formation of *iso*oleic acid during the hydrogenation of oil has been ascribed to the presence of finely divided metals acting as catalysts through the first creation of conjugated state in the linolic acid residue and then to partial hydrogenation of the latter.

It is well known that linseed oil dries quicker in contact with metallic driers than it does by itself and the nature of drier, temperature, humidity and nature of light exercise a profound influence in the drying process. Polymerisation, oxidation and decomposition, sometimes simultaneous and sometimes in stages, occur with the result that finally we get the linoxyn skin (Wolff, *Farben-Ztg.*, 1921, **26**, 2851 ; Friend, *J. Chem. Soc.*, 1917, **111**, 162 ; Genthe, *Z. angew. Chem.*, 1906, **19**, 2087 ; Sabin, *Ind. Eng. Chem.*, 1911, **3**, 84 ; De Waeb. *J. Soc. Chem. Ind.*, 1920, **39**, 49). The time of drying is further shortened if instead of raw oil we take boiled oil *i.e.*, linseed oil blown with air, which gives the appearance of boiling, in contact with catalysts, technically known as driers at 100-200°—the temperature depending on the drier used. (Rhodes and Wirt, *Ind. Eng. Chem.*, 1923, **15**, 1135 ; 1924, **16**, 960 ; Fokin and Eibner, *Chem. Umschau*, 1925, **32**, 97 : Mackay and Ingle, *J. Soc. Chem. Ind.*, 1917, **36**, 319).

During boiling there is always slight decomposition of glyceride and the notable changes consist in the reduction of iodine value and the refractive index. The present enquiry is confined to the chemical change if any, in the composition of linseed oil during "boiling" which is responsible for the acceleration in the time of its drying and for the matter of that, to that change in the oil which is responsible for the polymerisation, oxidation and decomposition already referred to. One of us (Goswami, *Science & Culture*, 1935, **1**, 283) had put forward views regarding such change. They are briefly the following :—

(*a*) There is transference of double bonds to conjugated state in the unsaturated fatty acid residues (*cf.* Kappelmeier, *Farben-Ztg.*, 1933, **38**, 77, 1018).

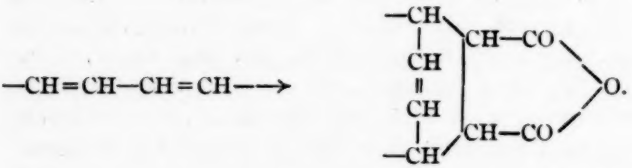
(*b*) There is formation of hydroxy fatty acid glyceride.

(*c*) There is fresh creation of double bonds in such hydroxy compounds as a result of elimination of elements of water.

(*d*) There is re-arrangement of double bonds of (*c*) to conjugated state and finally

(*e*) these changes are due mainly to metals in the finely divided state derived from the unstable metallic salts used as catalysts.

For the purpose of our experiments we prepared boiled oil under the conditions of the technical practice with regulated supply of air at 125° using various kinds of catalysts in various proportions We have been able to prove (*a*) and (*e*). A preliminary note regarding the above has been published (Goswami and Saha, *J. Indian Chem. Soc.*, 1937, **14**, 116). We have taken help of the well known reaction of maleic anhydride with compounds containing conjugatad double bond.



This reaction has already been applied in elucidating the constitution of eleostearic acid found in tung oil which dries faster than linseed oil when used alone owing to the presence of glyceride of the acid in the former oil. Thus eleostearic acid has been given the following formula :—

$$CH_3(CH_2)_3{\cdot}CH{=}CH{-}CH{=}CH{-}CH{=}CH(CH_2)_7{\cdot}COOH$$

Kaufmann and Baltes (*Fett. Seifen*, 1936, **43**, 93) have now evolved out a method based on this for the valuation of Tung oil which is always hopelessly adultrated and calculated maleic anhydride absorption value which they call Diene value, according to the following formula :—

$$\frac{a-b}{c} \times 1{\cdot}269$$

where a = no. of c.c. of $N/10$-NaOH required to neutralise 10 c.c. of 1% solution of maleic anhydride in acetone in blank experiment ; b = number of c.c. of $N/10$-NaOH required to neutralise the excess of maleic anhydride which remains when the oil is treated with it in sealed tube ; c = wt. of oil in g.

We have taken advantage of this method in establishing the presence of conjugated double bonds in boiled linseed oil.* The results obtained are given in the form of curves (*vide* Fig. 1-6).

The results with finely divided nickel although not very promising, support the view that the finely divided metals are responsible for the shifting of double bonds in the creation of conjugated state in linseed oil. They also throw light on the mysterious formation of *iso*oleic acid during the process of hydrogenation of oils in which finely divided nickel is used, presumably it is formed from the linolic acid residue according to the following way :

$$CH_3\cdot(CH_2)_4\cdot CH=CH\cdot CH_2-CH=CH\cdot(CH_2)_7-COO-$$

$$\longrightarrow CH_3\cdot(CH_2)_5\cdot CH=CH-CH=CH\cdot(CH_2)_7-COO-$$

$$\longrightarrow CH_3\cdot(CH_2)_5-CH-CH-CH-CH\cdot(CH_2)_7-COO-$$
$$\qquad\qquad\qquad\quad |\qquad |\qquad |\qquad |$$
$$CH_3\cdot(CH_2)_4-CH_2-CH=CH-CH_2\cdot(CH_2)_7-COO-$$
(*iso*leic acid residue).

Hilditch and Vidyarthi (*Proc. Roy. Soc.*, 1929, **A, 122**, 552) have made experiments to throw light on the subject. They hydrogenated methyl oleate in presence of nickel and showed the presence of *iso*oleic acid. But the methyl oleate they took contained, as they themselves have mentioned, methyl linoleate so their view that the *iso*oleic acid found in their experiments has been derived from oleic acid seems to be untenable. Their explanation of the formation is the following :

$$\longrightarrow \begin{array}{l} CH_3\cdot(CH_2)_7-CH=CH-(CH_2)_7-COO- \\ CH_3\cdot(CH_2)_7-CH_2-CH_2-(CH_2)_7-COO- \\ CH_3\cdot(CH_2)_6-CH=CH-(CH_2)_8-COO- \end{array}$$

In view of the evidence of migration of double bonds induced by finely divided metals, it seems to us that our explanation is more plausible one.

Our object in undertaking the present work has been

(1) to give a true picture of the chemical change which occurs when linseed oil is boiled,

(2) to explore the possibility of converting non-drying and semi-drying oils into drying oils,

(3) to bring the whole boiling operation as also the valuation of boiled oil under chemical control,

(4) to find out the solution of the *iso*oleic acid problem.

* Bruce and Denly (*Chem. Ind.*, 1937, **56**, 937) have suggested after determining Diene value of blown linseed oil and hydroxylated oils according to Ellis and Jones' method (*Analyst*, 1936, **61**, 812) that the value is affected by hydroxyl group. This aspect has been examined by Pelikan and Mikusch (*Oil and Soap* 1937, **14**, 209) who have shown conclusively that the determination of Diene value by Kaufmann and Baltes' method (*loc. cit.*) is not affected by hydroxyl group whereas Ellis and Jones' method is defective in this respect.

FIG. 1.

0·2 % *Nickel.*

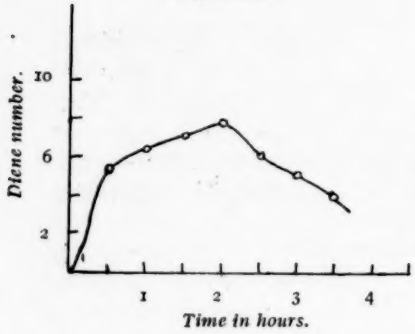


FIG. 2

2% *Nickel oleate.*

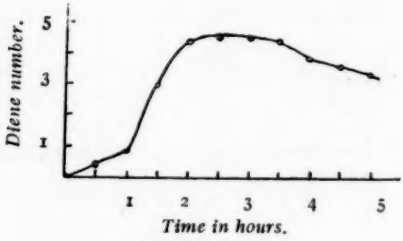


FIG. 3.

2% *Nickel.*

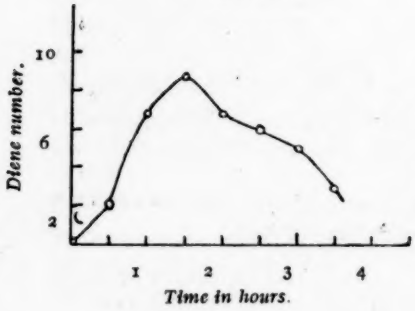


FIG. 4.

2% *Nickel rosinate.*

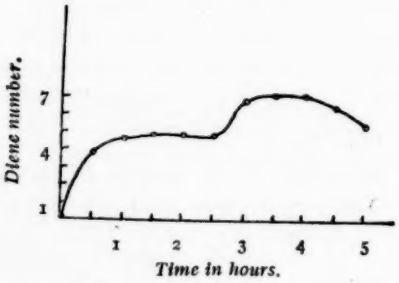


FIG. 5.

2% *Manganese oleate.*

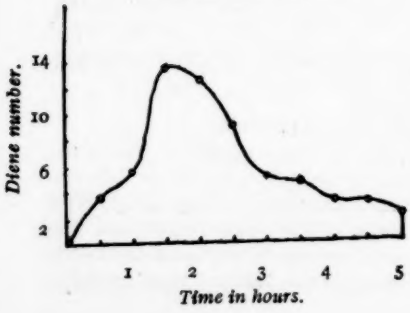
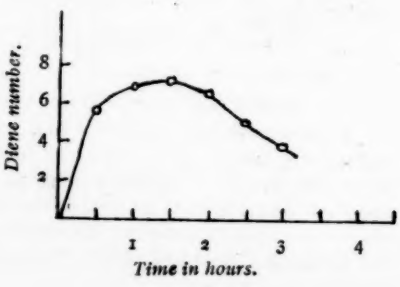


FIG. 6.

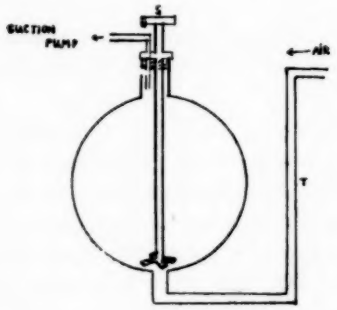
0·1% *Nickel.*



EXPERIMENTAL

The oil mixed with catalyst was taken in the round bottom flask. A regulated supply of air was drawn through the tube "T" which was conected with a gasometer by a suction pump. The tube "T" was connected at the bottom of the flask only to have an efficient stirring, although a separate stirrer "S" was placed in the flask. The flask was placed in an oil-bath, the temperature of which was kept at 125° Samples were drawn at every half an hour and their Diene values were found out in the following way. The oil was mixed with 1% solution of maleic anhydride and heated in a sealed tube for 20 hours; side by side a blank experiment in which the corresponding quantities of acetone solution of maleic anhydride were heated in a sealed tube for the same time was made. Both the solutions were then titrated against standard alkali to find out the amount of maleic anhydride taken up by the oil and the Diene values were calculated according to Kaufmann's formula. The following results are obtained.

FIG. 7.



Catalyst.	P.C.	½ hour.	1 hour.	1½ hours.	2 hours.	2½ hours.	3 hours.	3½ hours.	4 hours	4½ hours.	5 hours.
Mn-oleate	2	3·6	5.6	13·38	12·2	8·9	5·3	4·7	3·5	3·2	2·5
Ni- „	2	0·5	0·82	3	4·4	4·5	4·6	4·5	3·9	3·7	3·5
Ni-rosinate	2	3·9	4·7	4·7	4·8	4·9	6·4	6·4	6·8	6·1	6·1
Nickel	2	2·1	6·9	8·9	6·9	6·1	5·1	2·8	—	—	—
Nickel	0·2	5·5	6·1	7	8·9	6	5·2	4	—	—	—
Nickel	0·1	5·9	7·2	7·3	6·9	5·2	4·1	3·9	—	—	—

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VITAMIN C AND TOXINS. PART I. THE EFFECT OF VITAMIN C AND OTHER REDUCING SUBSTANCES ON DIPHTHERIA AND TETANUS TOXINS *IN VITRO*.

By Baidyanath Ghosh and B. C. Guha.

The effect of vitamin C, glutathione, cysteine and hydroquinone on diphtheria and tetanus toxins *in vitro* has been investigated. The inactivating effect of ascorbic acid is differentiated from the inactivation due to lowering in *p*H

The anti infective properties of vitamin C have been the subject of investigation in different laboratories for some time. It has been shown that guinea-pigs kept on a ration deficient in vitamin C are less resistant to diphtheria toxin than normal animals (Jungeblut and Zwemer, *Proc. Soc. Biol. Med.*, 1935, **32**, 1229; Jungeblut, *ibid.*, 1935, **32**, 1039). King and Menton (*J. Nutrition*, 1935, **10**, 122, 141; *ibid.*, 1935, **9**, proc. II) have claimed that the resistance of guinea-pigs against diphtheria toxin is increased by administration of large doses of ascorbic acid given *per os* or by injection. Others (Widenbauer and Saretz, *Klin. Woch.*, 1936, **15**, 1131; Polonyi, *Wien. Med. Wchnesch.*, 1935, **85**, 685) have reported the inactivation of diphtheria toxin *in vitro* by vitamin C. Sigal and King (*J. Pharm. Expt. Therap.*, 1937, **59**, 468), on the other hand, have stated that neutralised vitamin C is incapable of detoxicating diphtheria toxin *in vitro* and they conclude that the inactivation of the toxin by unneutralised ascorbic acid is probably due to the acidity of the solution and not due to the vitamin *per se*.

The present work was undertaken in order to study more fully the behaviour of diphtheria and tetanus toxins to vitamin C under different conditions and to throw light on the mechanism of inactivation. Since ascorbic acid is both a reducing substance and acidic in nature, it was considered desirable to study the behaviour of the toxins to other biologically active reducing substances and also to control the effect of the hydrogen ion concentration. In the present *in vitro* experiments we have studied the effect of vitamin C and of other reducing substances like glutathione, cysteine, hydroquinone, etc., and also the effect of p_H on the inactivation of the toxins. A preliminary note on this subject giving some main results has been published elsewhere (Ghosh and Guha, *Science and Culture*, 1937, **3**, 243).

EXPERIMENTAL.

Diphtheria and Tetanus Toxins and Ascorbic Acid.

A solution (1 c.c.) of the toxin in normal saline standardised to contain 1 m. l. d. of the toxin was mixed with 25 mg. of ascorbic acid and allowed to stand for 15 minutes at room temperature (27°) and the mixture injected subcutaneously into guinea-pigs weighing between 250 and 300 g. All the animals survived, remained healthy and gained in weight during the next two months—the period under observation—while the control animals died within 4 days as shown in Table I.

TABLE I.

Nature of toxin.	Dose.	Number of animals Treated.	Survived.	Died.
Diphtheria	1 m. l. d.	6	None	6
"	1 m. l. d. mixed with ascorbic acid (25 mg.)	6	6	None.
Tetanus	1 m. l. d.	6	None	6
"	1 m. l. d. mixed with ascorbic acid (25 mg.)	6	6	None

In the next series of experiment the effect of smaller quantities of ascorbic acid was investigated (Table II), which show that a small dose like 2 mg. is capable of inactivating the toxins.

TABLE II.

Toxin employed.	Ascorbic acid added to 1 m. l. d. toxin.	Number of animals Treated.	Survived.
Diphtheria	10 mg	4	4
"	5	4	4
"	2	4	4
Tetanus	10	6	6
"	5	4	4
"	2	4	4

Diphtheria and Tetanus Toxins and Reducing Substances other than Ascorbic Acid.

Since ascorbic acid is a strongly reducing agent it was considered possible that the mechanism of inactivation involves the reduction of the toxins. In order to test this view other reducing substances like glutathione, cysteine and hydroquinone were employed. These substances were found to be equally capable of detoxicating the toxins. 25 Mg. of these reducing substances (gluthathione, cysteine and hydroquinone) were mixed *in vitro* with the toxin and after standing at room temperature for 15 minutes injected into guinea-pigs, which showed no symptoms of toxicity subsequently. The detoxicating action of glutathione has been reported at about the same time by Vincent (*Compt. rend.*, 1937, **204**, 1693; Binet, Janlmes and Weller, *Compt. rend.*, 1937, **204**, 1761; Ghosh and Guha, *loc. cit.*) The results are given in Table III.

TABLE III.

	(*a*) Diphtheria toxin.			(*b*) Tetanus toxin.		
	Number of animals			Number of animals		
Substances mixed with 1 m. l. d. toxin.	Treated.	Survived.	Died.	Treated.	Survived.	Died
Glutathione (25 mg.)	6	6	None	6	6	None
Cysteine (25 mg.)	6	6	,,	6	6	,,
Hydroquinone (25 mg.)	6	5	1*	6	6	,,
1 m. l$_{\text{d}}$. toxin alone	6	None	6	6	None	6

* Died after 10 days.

Diphtheria and Tetanus Toxins and p_{H}.

The following experiments were undertaken to control the effect of p_{H}. It was found that diphtheria toxin has a p_{H} near about 6·8, while that of tetanus toxin lies between 7·2 and 7·4. On addition of reducing substances (ascorbic acid, glutathione, cysteine and hydroquinone), the p_{H} of the mixture goes down to 2·8 – 1·4 (Table IV).

TABLE IV.

	p_{H}.		p_{H}.
Diphtheria toxin	6·8	Tetanus toxin	7·2-7·4
,, +25 mg. ascorbic acid	2·8	,, +25 mg. ascorbic acid	2·8
,, +25 mg. glutathione	2·8	,, +25 mg. glutathione	2·8

TABLE IV (*contd.*).

	p_H			p_H
Diphtheria toxin+25 mg. cysteine	1·4	T. Toxin	+25 mg. cysteine	1·4
,, +25 mg. hydroquinone	3·5	,,	+25 mg. hydroquinone	3·5

In order to investigate whether simple lowering of the p_H of the toxin can effect detoxication, a drop or two of hydrochloric acid was added to the saline solution of the toxin so that the final p_H lay at about 2·8. It was found that the toxins were completely inactivated. Six animals injected with the acidified diphtheria toxin and 5 animals injected with the acidified tetanus toxin—all survived, while five control animals injected with toxin alone died within 3 days.

The effect of different hydrogen ion concentrations on the activity of the toxin was next studied. A large number of experiments were carried out with citrate-phosphate buffer (McIlvaine) varying in p_H from 4·6 to 6·8 and it was found that below p_H 5·0, both tetanus and diphtheria toxins are in activated, while above this p_H they remain active.

TABLE V.

	(*a*) Diphtheria toxin. Number of animals			(*b*) Tetanus toxin. Number of animals		
p_H.	Treated.	Survived.	Died.	Treated.	Survived.	Died.
4·6	6	6	None	—	—	—
5·0	6	6	,,	6	6	None
5·4	6	None	6	6	None	6
6·0	6	,,	6	—	—	
6·8	6	,,	6	6	None	6

Since at p_H 5·4 both the toxins are active, it was considered desirable to know whether ascorbic acid brought to p_H 5·4 by addition of sodium hydroxide would have any inactivating effect on the toxins at p_H 5·4. It is clear that if it is so, the detoxicating effect of vitamin C cannot merely be due to acidity but is probably due to the action of the vitamin *per se*. Ascorbic acid was, therefore, dissolved in saline or citrate-phosphate buffer at p_H 5·4 and caustic soda was added to bring the p_H back to 5·4, then the toxin was added, the final p_H was checked to be 5·4 and the mixture injected into animals. These experiments were carried out with toxin and all the animals so treated survived (Table VI). This would indicate that in the case of diphtheria toxin, ascorbic acid has some action apart from acidity—a view which is supported by Jungeblut (*J. Immunol.*, 1937, **33**, 203) in his experiments with tetanus toxin.

The effect of ascorbic acid raised to p_H 6·8 on the toxin was also studied to see if the vitamin will have any inactivating effect near the neutral zone, but with both the toxins the effect of the vitamin was found to be nil at this p_H.

TABLE VI.

	(a) Diphtheria toxin. Number of animals		
	Treated.	Survived.	Died.
Ascorbic acid at p_H 6·8	6	None	6
,, ,, ,, 5·4 ... ... (in citrate-phosphate buffer)	6	6	None
Hydrochloric acid at p_H 5·4	5	,,	5
Toxin at p_H 5·4 (in citrate-phosphate buffer) ...	6	,,	6
	(b) Tetanus toxin.		
Ascorbic acid at p_H 6·8	6	,,	6

CONCLUSION.

(1) The effect of vitamin C in different quantities on diphtheria and tetanus toxins *in vitro* was investigated and it was found that 2 mg. of the vitamin mixed with 1 m. l. d. of the toxin was able to cause inactivation.

(2) Similar inactivating effect was also observed with other reducing substances—glutathione, cysteine and hydroquinone.

(3) The effect of p_H on the toxin was studied and it was found that the lowering of p_H results in quick inactivation.

(4) Diphtheria toxin at p_H 5·4 is active but it is detoxicated at the same p_H in the presence of ascorbic acid. This indicates that the effect of ascorbic acid is at least partially due to the effect of ascorbic acid *per se* and not merely a p_H effect.

(5) At p_H 6·8 ascorbic acid has no inactivating effect on the toxin studied.

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VITAMIN C AND TOXINS. PART II. THE EFFECT OF THE ADMINISTRATION OF VITAMIN C TO GUINEA-PIGS INJECTED WITH DIPHTHERIA AND TETANUS TOXINS.

By Baidyanath Ghosh and B. C. Guha.

The effect of the injection and ingestion of ascorbic acid before and after injection with diphtheria and tetanus toxins is investigated and the question of immunity by vitamin C administration is discussed.

In the preceding communication (*J. Indian Chem. Soc*, 1938, **15**, 438) we have described the effect of vitamin C and other reducing substances on diphtheria and tetanus toxins *in vitro*. It has been shown that the inactivating effect of ascorbic acid in diphtheria toxin is not solely a p_H effect and is to be attributed at least partially to the action of the vitamin itself. This property of vitamin C led us to carry out certain experiments *in vivo* in relation to vitamin C and diphtheria and tetanus intoxication. The effect of the injection of the vitamin immediately before and after the injections of the toxins into guinea-pigs was studied. The effect of the injection of massive doses of the vitamin some time after the injection of toxins was also investigated. Further the protective action if any, of feeding or injecting with the vitamin for a period preceding the injection of the toxins was sought to be explored. The experiments were undertaken both as a sequel to our *in vitro* experiments and also in view of the claims of Greenwald and Harde (*Proc. Soc. Expt. Biol. Med.*, 1935, **32**, 1157) that ingestion of the vitamin confers protection on the guinea-pigs against subsequent injection of diphtheria toxin. King and Menten (*J. Nutrition*, 1935, **10**, 129, 141) have, of course, shown that in vitamin C deficiency guinea-pigs are more susceptible to infection but Jungeblut (*J. Immunol.*, 1937, **33**, 203) has found that ingestion of the vitamin does not confer complete protection against the effect of the injection of tetanus toxin. The subject obviously requires further investigation.

Experimental.

The diphtheria and tetanus toxins were standardised and their m.l.d's determined. Guinea-pigs weighing between 250 and 300 g. were taken as experimental animals. In the following experiments guinea-pigs injected with the toxin alone were always kept as controls and these died

in course of 3-5 days. Toxins were diluted with saline solution. Likewise, the reducing substances were dissolved in saline.

Injection of Ascorbic Acid immediately before and after Injection of the Toxins.

One m. l. d. of the toxin was injected, and ascorbic acid (100 mg.) in saline (1 c.c.) was injected within one minute after the injection of the toxin, and on a site remote from that of the toxin injection. There appears to be some protective action (Table I). Another reducing substance, cysteine, was injected in a similar manner, which however, unlike ascorbic acid, failed to protect the animals. Hydrochloric acid in saline (1 c.c. p_H 2·0). which was used as a control for the acidity of the ascorbic acid solution, when similarly administered, gave negative results. Neutralised ascorbic acid in saline at p_H 6·8 has so far failed to check the effect of toxin injection as was also observed in *in vitro* experiments (Ghosh and Guha, *loc. cit.*). The results are shown in Table I.

TABLE I.

Substances injected after 1 m. l. d. of toxin had been injected.	(a) Diphtheria toxin. Number of animals			(b) Tetanus toxin. Number of animals		
	Treated.	Survived.	Died.	Treated.	Survived.	Died.
Ascorbic acid (100 mg.)	8	6	2*	9	4	5
Cysteine (100 mg.)	5	None	5	5	None	5
Neutralised ascorbic acid (100 mg.)	4	,,	4	4	—	4
Hydrochloric acid	5	1	4	5	—	5
Toxin (m. l. d.) alone.	6	None	6	6	—	6

* Of these, one died after 12 days and the other after 16 days.

Injection of 100 mg. of ascorbic acid a minute before the toxin injection gave the following results (Table II), which again indicate a somewhat favourable effect of the injection of ascorbic acid.

TABLE II.

Toxin.	Number of animals		
	Treated.	Survived.	Died
Diphtheria	5	2	3
Tetanus	4	2	2

Delayed Injection of Massive Doses of Ascorbic Acid.

A delay in the injection of ascorbic acid (100 mg.) even half an hour after the toxin injection failed, however, to protect the animals. Repeated daily injections of ascorbic acid in massive doses for 3 to 4 days after the injection of the toxins also failed to save the animals from death (Table III).

TABLE III.

Toxin.	Period after which ascorbic acid was injected.	Amount of ascorbic acid injected daily.	No. of animals	
			Treated.	Died
	½ hour	100 mg.	3	3
	1 hour	100	2	2
	,,	250	2	2
A. Diphtheria	1 day	350	5	5
	,,	500	3	3
	,,	1000	2	2
	2 days	300-500	3	3
B. Tetanus	1 day	350-750	4	4

In the cases of animals injected with diptheria toxin subsequent injection after one day of 250 mg. of cysteine and of hydroquinone into two animals failed to exert any protective action. In the case of hydroquinone the animal died within 1 hour with shivering and convulsions. The cysteine-treated animal also succumbed.

The Effect of Previous Ingestion and Injection of Vitamin C.

Greenwald and Harde (*loc. cit.*) claimed that the ingestion of 10-20 mg. of ascorbic acid increased the resistance of guinea pigs to 1 m. l. d. of toxin after the 1st or 2nd day of feeding. We fed guinea-pigs daily 50 mg. of ascorbic acid for six days and on the 7th day 1 m. l. d. of the toxin was injected. Practically all the animals died within three days like the controls. Almost similar negative results were obtained when the animals were injected with 20 mg. of ascorbic acid daily for 6 days before toxin injection (Table IV). It seems that if some resistance is conferred on the animals, it can only be slight.

TABLE IV.

(a) Guinea-pigs fed with 50 mg. of ascorbic acid daily for 6 days prior to toxin injection.				(b) Guinea-pigs injected with 20 mg. of ascorbic acid in 0·9% saline daily for 6 days prior to toxin injection.			
Toxin used (1 m.l.d.).	Number of animals			Toxin used (1 m.l.d.).	Number of animals		
	Treated.	Survived.	Died.		Treated.	Survived.	Died.
Diphtheria	6	None	6	Diphtheria	6	2	4
Tetanus	6	1	5	Tetanus	6	None	6

Does the Previous Injection of Toxin-vitamin C Mixture increase Resistance against the Toxins?

That guinea-pigs which have survived for 5-7 weeks the injection of the toxin-vitamin mixture may develop some resistance against further injection of 2 m. l. d's of the toxin was claimed by Greenwald and Harde (*loc. cit.*). Negative results under similar circumstances were, however, obtained by us when 2 m. l. d's of the diptheria toxin were injected. In the cases where 1 m. l. d. of the diptheria toxin was used the majority of animals survived.

TABLE V.

Period in weeks between toxin-vitamin injection and fresh toxin injection.	Dose.	Number of animals		
		Treated.	Survived.	Died.
5	2 m. l. d's of diphtheria toxin.	4	None	4
5	1 m. l. d. of diphtheria toxin.	4	3	1

Another set of experiments was carried out to investigate whether three consecutive injections of the toxin-vitamin mixture previous to the injection of 2 m. l. d's of the diphtheria toxin can produce any immunity. No increased resistance or immunity was observed. In this case 1 m. l. d. of the diphtheria toxin mixed with 25 mg. of ascorbic acid was injected into guinea-pigs every fourth day; after 3 such toxin-vitamin injections 2 m. l. d's of the diphtheria toxin were injected into the animals. All the four animals, so treated, died within 4 days.

Discussion.

Although *in vitro* experiments show that vitamin C may have some specific inactivating effect on diphtheria and tetanus toxins (Ghosh and Guha, *loc. cit.*), the *in vivo* experiments do not favour the view that the vitamin confers any definite protection against diphtheria and tetanus toxins. The aciton of the toxins is apparently so quick and powerful that the possible action of the vitamin, which is seen when the vitamin is mixed with the toxins prior to injection, is nullified when the vitamin and the toxins are separately injected. There is some protective action shown by the vitamin if injected immediately before and after injection of the toxins and this cannot be due to local neutralisation of the toxins by the vitamin, as they are injected in different sites. It seems possible that in these cases the temporary rise in the acidity of the blood following ascorbic acid injection may be responsible for the detoxication of the toxins, which are very susceptible to the lowering of p_H. The fact that previous injection and ingestion of vitamin C during a fairly long period before toxin injection confers hardly any protection shows that the vitamin cannot be looked upon as a direct antitoxic agent. The previous injection of the vitamin-toxin mixtures also did not appear to increase the resistance of the guinea-pigs to these toxins. King and Menten (*loc. cit.*) have shown, however, that guinea-pigs deficient in vitamin C are more susceptible to diphtheria toxin. Abbasy, Hill and Harris (*Lancet*, 1936, *ii*, 1413) have also observed that rheumatism and tuberculosis cause depletion of vitamin C reserves of the body, which is apparently a result of the increased metabolism of vitamin C. It would seem, thorefore, that although vitamin C may not be a direct antitoxic agent, its deficiency would likely make an organism more susceptible to certain infections and aggravate some diseased conditions. Normally, therefore, vitamin C in liberal quantities should form part of the food as an indirect anti-infective agent. Further observations from this laboratory on the powerful inhibitory effect of vitamin C on the growth of certain bacteria *in vitro* also indicate the possibility that normal consumption of vitamin C may be helpful in guarding against bacterial infections. Further work in this line is, however, in progress.

Conclusion.

(1) Injection of ascorbic acid immediately before and after injection of 1 m. l. d. of the diphtheria and tetanus toxins appears to have some protective action on guinea-pigs.

(2) A delay in the injection of ascorbic acid failed to save the animals, even when massive doses of the vitamin were administered.

(3) Previous administration of ascorbic acid either orally or by injection failed to confer any definite protection against the toxin.

(4) Previous injection with the toxin-vitamin mixture also failed to confer any protection against diphtheria toxin injected subcutaneously.

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OBSERVATION ON THE RELATION BETWEEN PREGNANCY, SEX HORMONES AND THE VITAMIN C CONTENT OF THE TISSUES OF GUINEA-PIGS.

By Baidyanath Ghosh.

The effect of pregnancy and of the injection of a luteinising hormone on the onset of scurvy in guinea-pigs was investigated and, contrary to Bourne, no delaying effect was observed. Injection of luteinising and follicular hormones into virgin guinea-pigs did not appreciably alter the vitamin C content of the different tissues of the animals. Results of statistical analysis are given.

It was claimed by Bourne (*Nature*, 1935, **135**, 148) that injection of the homone preparation antuitrin-S into female guinea-pigs fed on a scorbutic diet prevented or delayed the onset of scurvy. As antuitrin-S stimulates the corpus luteum of the ovary, it was supposed that the luteal tissue was capable of synthesising vitamin C. It was also shown by Mouriquand and Schoen (*Compt. rend. Soc. Biol.*, 1933, **107**, 203) that gravid female guinea-pigs develop no or very slight scurvy when kept on a scorbutic diet. Further observations by Rohmer *et al* (*Nature*, 1934, **134**, 142; *Compt. rend. Soc. Biol.*, 1934, **116**, 1416; *ibid.*, 1935, **118**, 56) indicated that young infants upto an age of 5 months are capable of synthesising vitamin C. The conclusion was drawn that the embryo was able to synthesise vitamin C and that in the case of human subjects, this ability persisted for a short time after birth. Lately, Rohmer, Bezssonoff and Stoerr (*Compt. rend. Soc. Biol.*, 1936, **121**, 987) found a higher concentration of vitamin C in the cerebrospinal fluid of prematurely born infants than in that of normal infants, which they considered to lend support to their argument that the foetus synthesises vitamin C. This view is not supported by Mouriquand *et al* (*Compt. rend. Soc. Biol.*, 1936, **121**, 1005). Inconclusive results were obtained by Giroud *el al* (*ibid.*, 1936, **121**, 1061). Laporta and Renaldi (*Bull. soc. Ital. sper.*, 1936, **11**, 141) do not support the view that corpus luteum or the foetus can synthesise vitamin C.

In view of these contradictory results the present experiments were carried out and the behaviour of pregnant guinea-pigs and also of guinea-pigs injected with antuitrin-S on a scorbutic diet was investigated approximately by the method of Bourne. Further, the action of luteinising hormone (antuitrin-S) and of the follicular hormone (theelin) on the concentration of vitamin C in different organs like liver, kidney, spleen, small intestine and adrenal glands of female guinea-pigs has been investigated in order to explore whether these powerful sex-hormones have any relation to the synthesis of vitamin C in the body. The question seemed worthy of

study as a possible relation between vitamin C and sex hormones is indicated by the work of Bourne and some other investigators and also by the fact that the corpus luteum and the anterior pituitary body are relatively very rich in vitamin C.

EXPERIMENTAL.

Three sets of female guinea-pigs were kept on a scorbutic diet consisting of powdered gram (20 parts) and oats (80 parts), cod liver oil, salt and milk autoclaved for half an hour at 12 lb pressure. The three sets of animals consist of:—(1) virgin guinea-pigs kept as controls; (2) virgin guinea-pigs injected subcutaneously with 50 rat units (0·5 c.c.) of antuitrin-S (Parke Davis & Co) daily till they died; (3) pregnant guinea-pigs.

TABLE I.

No.	Body weight.	Date of beginning the expt.	Date of death.	Period of survival on scorbutic diet.
A. Controls.				
1	230 g.	11-11-37	9-12-37	28 days.
2	200	,,	8-12-37	27
3	215	,,	24-12-37	43
4	210	,,	24-12-37	43
5	220	,,	,,	43
B. Treated with antuitrin-S.				
1	205	,,	22-12-37	41
2	180	,,	21-12-37	40
3	190	,,	22-12-37	41
4	170	,,	2-12-37	21
5	250	,,	3-12-37	22
C. Pregnant.				
1	300	,,	7-12-37	26
2	260	,,	Survived*	...
3	260	,,	20-12-37	39
4	230	,,	Survived* died on 29-12-37.	...
5	335		Survived*	...
6	325	,	7-12-37	26
7	280		Survived*	...
8	285	,,	24-12-37	43

* Survived after 24-12-38, when all the controls died.

Another two sets, one consisting of virgin female guinea-pigs and the other consisting of pregnant guinea-pigs in their advanced stage of pregnancy were experimented on.

TABLE II.

A. Pregnant.

No.	Body weight.	Date of beginning the expt.	Date of death.	Period of survival on scorbutic diet.
1	422 g.	2-2-38	4-3-38	30 days.
2	447	,,	15-3-38	30
3	467	,,	23-3-38	49
4	370	,,	28-3-38	44
5	380	,,	27-3-38	53

B. Controls.

1	260	2-2-38	12-3-38	40
2	270	,,	7-3-38	35
3	240	,,	20-3-38	48
4	250	,,	20-3-38	48

Effect of Antuitrin-S and Theelin on Vitamin C Concentration in the Organs of Normal Female Guinea-pigs.

Three sets of female virgin guinea-pigs were taken, of which one set of 6 animals was injected every day with 0·5 c.c. (50 rat units) of antuitrin S. The second set of 6 animals was treated with 0·25 c.c. (50 international units) of theelin, while the third set of animals was kept as control. For seven days the subcutaneous injection of antuitrin-S and theelin were continued. On the eighth day each animal was killed by a blow on the head and in different organs the vitamin C values were estimated by the usual titrimetric technique with 2:6-dichlorophenol-indophenol (Ghosh and Guha, *J. Indian Chem. Soc.*, 1935 **12**, 30).

TABLE III.

A, Theelin (50 international units.

No.	Body wt.	Ascorbic acid in mg. per 10 g. of liver.	3 g. of kidney.	1 g. of spleen	5 g. of adrenal +small intestine.
1	285 g.	0·65	0·12	0·340	0 33
2	240	0·64	0·14	0·265	0 31
3	255	0·68	0·18	0·220	0·27
4	190	0·80	0·27	0·380	0·28
5	225	0·88	0·11	0·230	0·19
6	200	0·90	0·22	0·325	0·40
	Average	0·76	0·17	0·29	0·30

B. Antuitrin-S (50 rat units).

1	200	0·90	0·20	0·320	0·13
2	280	0·70	0·13	0·276	0·22
3	255	0·80	0·18	0·216	0·31
4	235	0·93	0·23	0·360	0·23
5	250	0·87	0·17	0·200	0·19
6	170	0·85	0·14	0·206	0·28
	Average	0·84	0·18	0·263	0·23

C. Control.

1	240	0·94	0·23	0·265	0·38
2	265	0·95	0·18	0·180	0·30
3	225	0·91	0·18	0·172	0·30
4	245	0·96	0·17	0·300	0·22
5	195	0·63	0·17	0·345	0·22
6	195	0·93	0·16	0·310	0·34
	Average	0·89	0·18	0·262	0·29

Statistical Note.

By K. C. Basak.

It is clear from Table I that antuitrin-S has failed to produce any improvement. The animals in the control group lived for 32·8 days on an average, while the corresponding average life of those treated with antutrin-S was 33 days.

If we take the period between 11-11-37 and 24-12-37 as the period of experiment and compare the pregnant group with the control group we get the following data.

	Died.	Survived.	Total.
Control	... 5	...	5
Pregnant	... 4	4	8
Total	... 9	4	13

The probability of such an event arising out of chance alone is given by

$$P = \frac{28}{143} = 19{\cdot}6\%.$$

With such high probability for chance occurrence we are to conclude that pregnancy does not make any significant difference,

In the second set of experiments (Table II) the behaviour of the pregnant animals is probably inferior to that of the controls. The average lives of the pregnant and the control animals are 28·6 days and 40·8 days respectively.

The mean ascorbic acid contents in liver, kidney, spleen and small intestine in each of the three sets of experiments, as calculated from Table III, are shown in the following table :—

Mean Ascorbic Acid in mg.

Expt.	Liver (per 10 g).	Kidney (per 3 g.).	Spleen (per g.).	Small intestine (per 5 g.).
A	0·76	0·17	0·29	0·30
B	0·84	0·18	0·26	0·23
C	0·89	0·18	0·26	0·29

The following analyses of variance have been worked out to test the significance of the differences of the means in the three experiments,

Analysis of variance.

	Source of variation.	Degree of freedom.	Sum of square.	Mean square.
Liver	Between expt.	2	0·0545	0·0273
	Within expt.	15	0·1852	0·0123
	Total	17	0·2407	
Kidney	Between expt.	2	0·0002	0·0001
	Within expt.	15	0·0296	0·0020
Spleen	Between expt.	2	0·0038	0·0019
	Within expt.	15	0·0686	0·0046
	Total	17	0·0724	
Small intestine	Between expt.	2	0·0187	0·0094
	Within expt.	15	0·0654	0·0004
	Total	17	0·0841	

In two cases the mean square between experiments, is greater than that within experiments, but the ratio is not significant. Hence injections of antuitrin-S and theelin has not given results significantly different from those produced in the control group.

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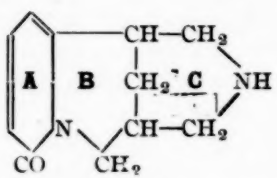
EXPERIMENTS ON THE SYNTHESIS OF CYTISINE. PART I. SYNTHESIS OF ETHYL 3 : 5-DICARBETHOXYPYRONE-6-ACETATE AND THE CORRESPONDING PYRIDONES.

By S. K. Mitra.

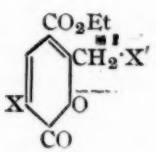
The synthesis of ethyl 3 :5-dicarbethoxypyrone-6-acetate has been effected. It has been condensed with ammonia and primary amines giving rise to corresponding pyridones. In course of formation of pyridones, one of the carbethoxy groups in the β-position of the nitrogen atom is hydrolysed and the ammonium form of the pyridone results. β-Aminopropionic ester does not condense with the above mentioned pyrone. On the other hand, ethyl 3-cyano-5-carbethoxypyrone-6-acetate condenses with β-amino-propionic ester. The resulting product furnishes a hydroxymethylene derivative with ethyl formate.

The alkaloid cytisine occurring in the seeds of *Cytisus Laburnum* and a large number of leguminosæ was first isolated in a pure state by Husemann and Marme (*Edin. Med. J.*, 1862, **7**, 908, 1025) and a considerable amount of work has been done by Partheil * and Freund † and his collaborators regarding its constitution. The present investigation was undertaken to synthesise cytisine which has been assigned structure (I).‡

The fundamental idea underlying the attempt is to effect a synthesis of the pyridone ring A with substituents at the positions '1' and '6' and then to form the rings B and C. For the synthesis of the desired pyridone, the corresponding pyrone (II) was obtained by condensing sodio acetone dicarboxylic ester with ethoxymethylene malonic ester (X=X′=CO_2Et; *cf.* Simonsen, *J. Chem. Soc.*, 1908, **93**, 1022).



(I)

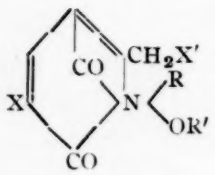


(II)

* *Arch. Pharm*, 1892, **230**, 448; 1894, **232**, 167; *Ber.*, 1890, **23**, 3202; 1891, **24**, 635.

† *Ber.*, 1901, **34**, 605; 1904, **37**, 16; 1906, **39**, 814; *Arch. Pharm.*, 1918, **256**, 33; Ewins, *J. Chem. Soc.*, 1913, **103**, 97.

‡ Späth, *Monatsh.*, 1919, **40**, 1593; *Ber.* 1932, **65B**, 1526; *Ber.*, 1933, **66B**, 1338; *Ber.*, 1938, **71**, 721. Ing, *J. Chem. Soc.*, 1932, 2778; 1933, 504; 1931, 2195.



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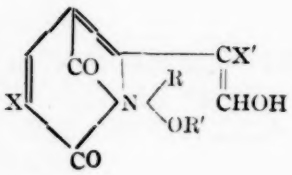

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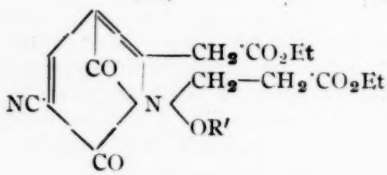

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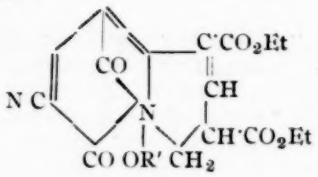

(VI)

The pyrone readily furnished the pyridone (III, X = X′ = CO_2Et; R′ = H ; R = H or alkyl) in conformity with the observations of Simonsen (*loc. cit.*). For obvious reasons the pyridone (ammonium form) could not be esterified by usual processes. The isomeric ether (III, X = X′ = CO_2Et, R = H or any group ; R′ = alkyl) could, however, be isolated by the silver oxide method (Irvine, *J. Chem Soc.*, 1908, **93**, 1608). Attempted synthesis of pyridone (III, R′ = H ; R = $CH_2{\cdot}CH_2{\cdot}CO_2Et$; X, X′ = CO_2Et) from the interaction of pyridone (III, R′ = R = H ; X = X′ = CO_2Et) and β-chloropropionic ester failed. β-Aminopropionic ester could not be condensed with the same pyrone. The latter, however, was condensed with β-aminoethyl alcohol furnishing the pyridone (III, X = X′ = CO_2Et ; R′ = H ; R = $CH_2{\cdot}CH_2{\cdot}OH$). The chloro compound (III, X and X′ = CO_2Et; R′ = H; R = $CH_2{\cdot}CH_2{\cdot}Cl$) was isolated by the action of thionyl chloride on the above pyridone. It is important to mention in this connection that the hydroxyl group attached to the nitrogen atom of the pyridone in the ammonium form did not take part in chlorination, since under similar conditions chloropyridone (III, R = H; OR′ replaced by Cl ; X = X′ = CO_2Et) could not be isolated. The cyano compound (III, X = X′ = CO_2Et ; R′ = H; R = $CH_2{\cdot}CH_2{\cdot}CN$) could not be isolated by the action of potassium cyanide on the chloro compound. The synthesis of the same cyano compound from the corresponding bromo compound could not also be effected; the reaction with potassium cyanide furnished the pyridone (III, X = X′ = CO_2Et ; R′ = $CH{:}CH_2$). Considering the poor yield of the pyrone (II, X = X′ = CO_2Et) it was thought desirable to replace ethoxymethylene malonic ester with ethoxymethylene

cyanoacetic ester. The condensation, however, instead of following the line suggested by Simonsen, gave rise to the cyanopyrone (II, X=CN; X′=CO_2Et). This compound furnished the pyridone (III, X=CN; X′=CO_2Et; R=R′=H). The pyridone thus obtained could also be alkylated by the silver oxide method. The action of methylamine on this cyanopyrone is noteworthy. In equivalent proportions, it furnished the cyanopyridone (III, R=Me; R′=H; X=CN; X′=CO_2Et), but in excess it gave rise to an amide (III, X=CN; X′=CONHMe; R=Me; R′=H). The ethyl ether of the amide on condensation with ethyl formate furnished a compound giving colouration with $FeCl_3$ characteristic to β-ketonic esters, probably the hydroxymethylene compound (IV, X=CN; X′=CONHMe; R′=Et), thus proving the presence of a methylene group attached to the position 6 of the pyridone ring and consequently of the pyrone (II). β-Aminopropionic ester, however, condensed with the cyanopyrone giving rise to the desired pyridone (V), the ethyl ether of which was condensed with ethyl formate. The resulting hydroxymethylene compound (VI) could not be converted by *cyclo*-dehydration to (VII) with phosphorus pentoxide in ether suspension. The work, however, is in progress.

EXPERIMENTAL.

Ethyl 3:5-Dicarbethoxy-α-pyrone-6-acetate (II, X=X′=CO_2Et).—Ethyl acetonedicarboxylate (10 g.) was added to a cooled solution of alcohol (50 c. c.) containing sodium (2 g.). Ethoxymethylene malonic ester (10 g.) dissolved in alcohol (25 c. c.) was added to the mixture. The mixture was then left for 2 hours at 0°, diluted with excess of water and extracted with ether. The aqueous layer was acidified and extracted with benzene. The oil obtained on removal of benzene was heated to 160°/12 mm., till no more could be distilled off The residue furnished light yellow plates from alcohol or acetic acid, m. p. 106°. (Found: C, 55·0; H, 5·3. $C_{15}H_{18}O_8$ requires C, 55·2; H, 5·5 per cent).

Ethyl 2-Keto-3-carbethoxy-5-carboxy-1:2-dihydropyridine-6-acetate (III, X=X′=CO_2Et; R=R′=H).—The pyrone (II, X=X′=CO_2Et; 1g.)was treated with aqueous ammonia (4 c.c. of 26%) and left at the room temperature (15°). After 6 hours the mixture was diluted with water to three times the original volume and acidified. The precipitated material on washing with alcohol furnished colourless needles from acetic acid, m. p. 223°. (Found : N, 4·9. $C_{13}H_{15}O_7N$ requires N, 4·7 per cent).

The pyridine when freshly prepared could be dissolved in a solution of

sodium bicarbonate with evolution of carbon dioxide but on keeping for sometime it did not dissolve unless boiled with a solution of caustic soda. Obviously, the freshly precipitated material was the pyridone carboxylic acid which on keeping quickly passed on to the ammonium form. It gives rose-red colouration with ferric chloride.

Ethyl 1-(β)-H*ydroxyethyl-2-keto-3-carbethoxy-5-carboxy-1:2-dihydro-pyridine-6-acetate* (III, R=X; X′=CO_2Et; R=$CH_2{\cdot}CH_2OH$; R′=H).—The pyrone (2·5 g.) in ethyl alcohol (40 c.c.) was mixed with β-hydroxyethylamine (1 c.c.). The mixture was boiled under reflux (5 hours) till colourless flakes of β-hydroxyethylammonium salt of the desired pyridone-carboxylic acid, m. p. 189°, separated. (Found: N, 7·0. $C_{17}H_{26}O_9N_2$ requires N, 6·96 per cent).

It was dissolved in minimum quantity of water and acidified with dilute hydrochloric acid. The seperated material gave colourless flakes of the pyridone acid from alcohol, m. p. 109°. (Found : N, 4·1. $C_{15}H_{19}O_8N$ requires N, 4·1 per cent).

Ethyl 1-(β)-*Chloroethyl-2-keto-3-carbethoxy-5-carboxy-1:2-dihydropyridine-6-acetate* (III, X=X′=CO_2Et; R=$CH_2{\cdot}CH_2Cl$; R′=H). — The pyridone (III, X=X′=CO_2Et; R=$CH_2{\cdot}CH_2OH$; R′=H; 2 g.) was dissolved in thionyl chloride (5 c.c.) and boiled under reflux (4 hours) till no more hydrogen chloride evolved. After removal of thionyl chloride, the product was poured into ice and crystallised from alcohol, m. p. 115°. (Found : C, 49·73; H, 5·0. $C_{15}H_{18}O_7NCl$ requires C, 50·0; H, 5·0 per cent).

Ethyl-1-Vinyl-2-keto-3 - carbethoxy- 5-carboxy-1:2-dihydro - pyridine-6-acetate (X=X′=CO_2Et; R=$-CH=CH_2$; R′=H).—The pyridone (III, X=X′=CO_2Et; R = $CH_2{\cdot}CH_2OH$; R′=H; 1 g.) was treated with phosphorus pentabromide (4 g.) under ether (100 c. c.). The mixture was boiled under reflux (2 hours). The alcoholic solution of the material obtained on removal of ether was treated with an aqueous solution of potassium cyanide (2 g.) in water (5 c. c.). The mixture was boiled under reflux (1 hour). The alcohol was removed under reduced pressure and acidified. It was crystallied from alcohol, m. p. 130-33°. (Found : N, 4·3. $C_{15}H_{17}O_7N$ requires N, 4·3 per cent).

Ethyl 3-Cyano-5-carbethoxy-α-pyrone-6-acetate (II, X=CN; X′=CO_2 Et.)—Ethoxymethylene cyanoacetic ester (8 g.) in alcohol (20 c.c.) was added to ethyl acetonedicarboxylate (10 g) dissolved in alcohol (40 c.c.) containing sodium (2 g.) and cooled to 0°. The sodium compound, separating on keeping the mixture for 12 hours, was filtered and washed with ethyl alcohol.

The aqueous solution of the precipitate on acidification liberated the pyrone, which furnished colourless needles from alcohol or acetic acid, m. p. 142°. (Found : C, 55·9; H, 4·6; N, 5·1. $C_{13}H_{13}O_6N$ requires C, 55·9; H, 4·6; N, 5·02 per cent).

Ethyl 2-Keto-3-cyano-5-carboxy-1:2-dihydropyridine-6-acetate (III, X = CN; X′ = CO_2Et; R = R′ = H).—The pyrone (III, X = CN; X′ = CO_2Et; 1 g) was dissolved in aqueous ammonia (10 c.c. of 26%) and kept for 12 hours at room temperature (28°). The mixture was diluted and acidified. The material thus precipitated furnished colourless needles from acetic acid, m. p. 260° (decomp). (Found : N, 11·3. $C_{11}H_{10}O_5N_2$ requires N, 11·2 per cent).

Ethyl 1-Ethyl-2-keto-3-cyano-5-carboxy-1:2- dihydropyridine -6- acetate (III, X = CN; X′ = CO_2Et; R = Et; R′ = H).—The pyrone (II, X = CN; X′ = CO_2Et; 1g.) was dissolved in aqueous solution of ethylamine (10 c. c. of 33% solution) and kept for 12 hours at room temperature (28°). The mixture on dilution followed by acidification furnished the desired pyridone which was isolated as colourless needles from acetic acid, m. p. 152°. (Found : N, 9·9. $C_{13}H_{14}O_5N_2$ requires N, 10·0 per cent). *Ethyl 1-Methyl-2- keto-3-cyano 5-carboxy- 1 : 2-dihydropyridine- 6- acetate* (III, X = CN; X′ = CO_2Et; R = CH_3; R′ = H).—The pyrone (II, X = CN; X′ = CO_2Et; 1g.) was dissolved in aqueous solution of methylamine (10 c.c. of 30%) and allowed to stand for 12 hours. It was isolated in the usual manner and crystallised from acetic acid, m.p. 210°. (Found: N, 11·0. $C_{12}H_{12}O_5N_2$ requires N, 10·6 per cent).

Ethyl 1-Methyl-2-keto-3-cyano-5-carboxy-1 : 2-dihydropyridine-6-acetate-methyl ether (III, X = CN; X′ = CO_2Et; R = R′ = CH_3).—The pyridone (III, X = CN; X′ = CO_2Et; R = R′ = CH_3; 1·2 g.) was added to a mixture of benzene (200 c.c.) and methyl iodide (10 g.) in which dry silver oxide (5 g.) was suspended. It was boiled under reflux 8 hours and filtered. The material obtained on removal of benzene furnished colourless needles from petroleum ether (b.p. 90-100°), m. p. 123°. (Found: N, 10·05. $C_{13}H_{14}O_5N_2$ requires N, 10·0 per cent).

Methyl Amide of 1-Methyl-2-keto-3-cyano-5-carboxy-1 : 2-dihydropyridine-6-acetic Acid (III, X = CN; X′ = $CONHCH_3$; R = CH_3; R′ = H).—The pyrone (II, X = CN; X′ = CO_2Et; 1 g.) was dissolved in aqueous solution of methylamine (40 c.c. of 33%) and kept (12 hours) at room temperature (28°). It was diluted and acidified. It furnished colourless needles from acetic acid, m. p. 285°. (Found: N, 16·7. $C_{11}H_{11}O_4N_3$ requires N, 16·8 per cent).

The condensation of benzaldehyde with the pyridone thus obtained in presence of zinc chloride could not be effected. It gives rose-red colouration with ferric chloride.

Methyl Amide of 1-Methyl-2-keto-3-cayno-5-carboxy-1:2-dihydropyridine-6-acetic Acid-ethyl ether (III, X=CN; X′=CONHCH$_3$; R=CH$_3$; R′=Et).—The amide mentioned above (0·5 g.) was taken in dry benzene (100 c.c.) containing ethyl bromide (10 c.c.). Dry silver oxide (3g.) was then suspended and the mixture boiled under reflux for 8 hours. It was then filtered and the residue was washed successively with warm benzene. The precipitate obtained on removal of benzene furnished colourless flakes from the same solvent, m p. 173°. It does not give colouration with ferric chloride. (Found: N, 14·9. $C_{13}H_{14}O_4N_3$ requires N, 15·2 per cent).

The amide described in the previous experiment (0·4 g) was dissolved in dry benzene (100 c.c.) containing emulsified sodium (1 g.). Ethyl formate (5 c.c.) was then added and the mixture was left (8 hours) at the room temperature (28°). Ethyl alcohol (10 c.c.) was then added and the mixture extracted with excess of water. The aqueous solution was shaken several times with ether to remove the last traces of benzene. It was then acidified and the hydroxymethylene derivative thus liberated was dissolved in ether by repeated extraction. The material obtained on removal of ether gave colouration with ferric chloride.

β-Aminopropionic Ester Hydrocholoride.—The preparation of the ester from succinimide by the action of sodium hypobromite as described by Van Dorp (*Rec. trav. chim.*, 1891, **10**, 4) was not satisfactory. A better yield was however obtained by substituting sodium hypochlorite for hypobromite. Chlorine (5 g.) was passed into water (100 c. c.) containing caustic soda (10 g.) at 0°. Succinimide (5 g.) was added and the mixture vigorously stirred. The solution was kept at 60-70° when evolution of gas commenced with gradual disappearance of the colour. It was acidified with hydrochloric acid and evaporated to dryness. The last traces of water were removed under reduced pressure. The residue (a mixture of sodium chloride and β-aminopropionic acid hydrochloride) was taken in ethyl alcohol (150 c. c.) containing alcoholic hydrochloric acid (10 c. c.). The vapour of alcohol (500 c. c.) was then bubbled through the mixture under reflux (2 hours). The alcoholic solution was filtered and the filtrate evaporated to dryness. The residue was dissolved in minimum quantity of alcohol from which the ester hydrocloride was isolated as glistening flakes, m.p. 58° on addition of benzene, yield 50%. (Found : N, 9·4. $C_5H_{12}O_2NCl$ requires N, 9·1 per cent).

Ethyl 1 *(β)-Carbethoxy-ethyl-2-keto-3-cyano-5-carboxy-*1 : 2*-dihydro-pyridine-6-acetate* (V).—β-Aminopropionic ester hydrochloride (3 g.) was dissolved in alcohol(50 c. c.) and treated with aqueous caustic soda to make it just alkaline. The pyrone (0·5 g.) was added to the mixture which was boiled under reflux for 15 hours). The product obtained on removal of alcohol under reduced pressure was treated with water ; the unchanged pyrone was filtered off ; the aqueous extract after acidification was allowed to stand (1 hour) at the room temperature (28°). The precipitate obtained on filtration was treated with an excess of cold aqueous solution of sodium carbonate and filtered. The insoluble material furnished colourless needles from methyl alcohol, m. p. 113°. (Found: C, 54·7; H, 5·0; N, 8·08. $C_{16}H_{18}O_7N_2$ requires C, 54·8 ; H, 5·1 ; N, 8·0 per cent).

The compound (1 g.) described above was dissolved in dry ether containing ethyl bromide (20 g.). Dry silver oxide (3 g.) was next suspended in the mixture and it was boiled under reflux (8 hours). On removal of ether the ethyl ether was obtained as an oil which did not give any colouration with ferric chloride. It was dissolved in dry ether (25 c. c.) containing ethyl formate (0·5 g.). The mixture was then slowly added to dry ether (25 c. c.) in which emulsified sodium (0·5 g.) was suspended. The mixture was left (8 hours) at the room temperature (28°) and treated with alcohol (1 c. c.). The mixture was extracted with water. The aqueous alkaline solution was acidified and repeatedly extracted with ether. The viscous material which could not be crystallised, obtained on removal of ether, gave with ferric chloride an intense red colouration characteristic of hydroxy-methylene acetic esters. The semi-solid mass was then dissolved in ether (10 c. c.) and the mixture boiled under reflux (3 hours) with phos-phorus pentoxide (1 g.). The compound was recovered unchanged.

My thanks are due to Prof. Samuel Smiles and Sir P. C. Ray for their kind interest in this work and to Prof. P. C. Mitter for facilities granted.

I also record my indebtedness to Mr. N. Ghosh, M.Sc., for carrying out microanalyses.

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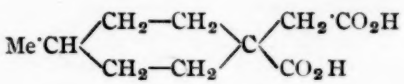
STRAINLESS MONOCYCLIC RINGS. PART II. SYNTHESIS OF 3-METHYL*CYCLO*HEXANE-1-CARBOXY-1-ACETIC ACID AND SEPARATION OF ITS ISOMERS.

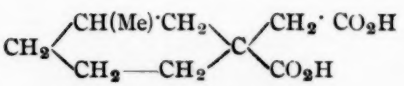
By Muhammad Qudrat-i-Khuda, Ashutosh Mukherji and Phanibhusan Banerji (in part).

The continuation of the work of the isolation of the isomeric *m* methyl*cyclo*hexane-succinic acid has yielded four isomeric acids. These melt at different temperatures, the first one melting at 88°, the second melts at 99°, whilst the other two melt at 168° and 157° respectively. The mixed m. p. of the different pairs indicates by a distinct lowering of the m. p. that they are separate compounds. Such derivatives as anhydrides and anilic acids, as also their imides lead to the same conclusion. The solubility of the ammonium salts of the acids in absolute alcohol also points out that they are not identical in character. The acid (m.p. 88°) when converted into its anhydride, gives a liquid anhydride which on decomposition with water gives two acids one melting at 99° and the other at 157°, this is an example of the interconversion of the acids by which the less stable one passes into its more stable analogue.

In part I of this series of papers (*J. Indian Chem. Soc.*, 1931, **8**, 277) the question of strain-free structure of *p*-methyl*cyclo*hexane ring has been discussed and definite evidence in favour of the same has been put forward. Uptil now no evidence could be obtained in support of a strain-free and consequently multiplanar configuration of *cyclo*hexanone ring itself, due probably, to the unchecked intramolecular rotation of the carbon atoms forming the ring. The moment substituents enter into more than one carbon atom forming the ring, the free intramolecular rotational motion of the different members constituting the ring is more or less checked. Thus it is possible for 4-methyl*cyclo*hexane-1-carboxy-1-acetic acid (I) to exhibit the isomerism by virtue of the geometrical position of the different members taking part in the formation of the molecule.



(I)

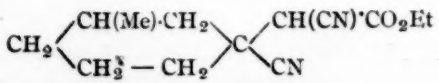


(II)

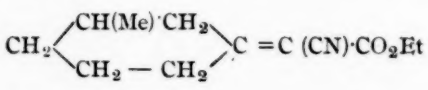
Goldschmidt and Grafinger (*Ber.*, 1935, **68**, 279) have questioned the truth of the above statement, saying that they were unable to isolate the four different forms of the acid (I). It is rather surprising that the experiments that were repeated a number of times by one of us (M.Q.K.) with the same result, should be found altogether wrong. The work has again been repeated in this laboratory and the results obtained fully support our original view (*cf. Nature*, 1936, **136**, 301).

Our work with *m*- and *o*-methyl*cyclo*hexanones were nearing completion when Desai, Hunter, Gholam Khan and Saharia (*J. Chem. Soc.*, 1936, 416) published a paper on the same subject but they could not get more than two isomers with either the *meta*- or the *ortho*-compounds. But our findings were different; we got results which go in favour of our original findings and in fact four distinctly different acids have been isolated in case of *m*-methyl*cyclo*hexane derivative, the results of which are described here. We repeated the experiments a number of times and always obtained the same result. It may be incidentally mentioned that the work did not prove to be a very plain sailing one, on the other hand it has taken several years of very patient work for its completion. We find that the difference in the position of the substituent in the *cyclo*hexane ring does not materially alter the behaviour of this ring from that of its *para*-isomer. In the boat shape configuration 3-methyl*cyclo*hexane-1-carboxy-1-acetic acid (II) will exist in two stereoisomeric modifications and in the chair shaped configuration again there will be possibility of the existence of two more stereoisomerides.

m-Methyl*cyclo*hexanone has been converted into the acid (II) through a series of reactions that are practically of the same nature as used in the previous case (Qudrat-i-Khuda, *loc. cit.*). Ethyl 3-methyl*cyclo*hexane-1-cyano-cyanoacetate (III) was obtained by the addition of potassium cyanide to ethyl 3-methyl*cyclo*hexylidinecyanoacetate (IV) in alcoholic solution. This ester was not isolated in pure condition, but was directly hydrolysed with concentrated hydrochloric acid. The dicyano ester (III) was found to be rather difficultly hydrolysable. Even after heating the acid mixture for nearly fifty hours, a small quantity of an acidic nitrogenous substance, a mixture of imides, could be isolated. As the product of hydrolysis a crude mixture was obtained, which consisted of several acids and the imide just referred to.



(III)



(IV)

The separation of the acids was effected through the ammonium salt, which dissolved completely in hot absolute alcohol A saturated alcoholic solution of the ammonium salts deposits on cooling part of it as a crystalline solid which dissolves in water completely and on acidification gives a mixture of acids melting indefinitely between 78-85°. On repeated crystallisation from benzene, this can be separated into two portions one melting at 99° (B) and the other which is more soluble in benzene melting at 88° (A). The acid (B) gives an anhydride melting at 45°. The portion of the ammonium salt soluble in cold water gave a small quantity of a solid imide, insoluble in water and melting indefinitely between 138-144°. The aqueous solution being acidified gave a solid acid, mixed with an oily acidic substance. It is rather difficult to effect a complete separation of this solid from the viscous mass. The solid has, however, been separated by benzene in which it does not dissolve to any appreciable extent, and melts at 168°(C). It gives a liquid anhydride. The benzene mother-liquor on being treated with petroleum ether gives some more of the solid acid, m. p. 168°. The benzene-petrol mother-liquor, on evaporation of the solvent, a gave a thick oily liquid which on esterification followed by hydrolysis gave a new acid (D) (along with some others) which is freely soluble in benzene and melts at 157°.

The ammonium salts of the pure acids A, B, C and D were found to have the following solubilities in alcohol:

Acids.	The amount of boiling alcohol required to dissolve 2 g of the ammonium salt.	The amount of the ammonium salt that crystallised out at ordinary temp. (28-30°)
A (m.p. 88°)	46 c.c.	0·2 g.
B (m.p. 99°)	72	0·7
C (m.p. 168°)	25	Nil.
D (m.p. 157°)	44	Only traces.

During the repetitions of these experiments, it has always been observed that the quantities of the isolable acids varied in different experiments, and it has not yet been possible to find an optimum condition for the best yield

of any of these acids. It has, however, been generally found that the quantity of the acids (A and B) depends somewhat on the duration of heating in the hydrolysis of the condensation product (III). When it was hydrolysed for about 30 hours, the quantity of this acid mixture was found to be only 15 g., whereas by increasing the period of heating to 46-50 hours, the yield increased to 70 g. for each 100 g. of the unsaturated ester (IV) employed in condensation with potassium cyanide in either of the experiments, other conditions remaining identical.

The isolation of the four isomeric acids in this case, and a comparison of these with the acids derived from *p*-methyl*cyclo*hexanone (m. p. 137°, 129°, 174° and 146°) decides conclusively that none of the isomers of the acid (I) is structurally identical with those now obtained.

The liquid anhydride of the acid A (m. p. 88°), when heated with water gave a mixture of acids from which an acid, m. p. 99° and another, m. p. 157°, have been isolated, but the change does not take place by heating (A) alone with water or with hydrochloric acid. It is perhaps this tendency of the less stable acid to pass over to its more stable analogue, that causes the difference in the total yield of the acids, as referred to above.

It also occurred to us that some of these acids might be eutectic mixtures of the others, but a comparative examination of the mixed m. p of the acids and those of their derivatives, left no room for doubts regarding the individuality of all the four acids. The acid (A) is much more soluble in cold benzene than the acid (B) and the acid (D) is extremely soluble in the same solvent in the cold, while (C) does not dissolve in it even when boiled. The anilic acid of (D) is soluble in cold benzene, whereas the other anilic acids are practically insoluble in the same solvent. The imide of acid (A) melts at 138°, while that of the acid (B) melts at 91°, which suggests that the acid (A) may not be an impure mixture of acid (B). Many attempts were made to crystallise mixtures of different acids, two at a time, but never could an eutectic mixture be obtained from these experiments.

TABLE I.

M. p. of the acids.			Mixed m p. of the acids.		
A	...	88°	A & B	...	92-93°
B	...	99°	A & D	...	89-91°
C	...	168°	A & C	...	125-130°
	...	157°	B & D	...	101°
			B & C	...	118-122°
			C & D	...	160°

TABLE II.

M. p. of the pure anilic acids.			Mixed m. p. of the anilic acids.		
W from A	...	166°	W & X	...	160°
X from B	...	180°	W & Y	...	145°
Y from D	...	171°	W & Z	...	158°
Z from C	...	173°	X & Y	...	150°
			X & Z	...	156-57°
			Y & Z	...	158°

TABLE III.

M. p. of the imides.			Mixed m. p. of the imides.		
M from A	...	138°	M & N	...	89-90°
N from B	...	91°	M & O	...	167-171°
O from C	...	183°	M & P	...	153-155°
P from D	...	176°	N & O	...	138-140°
			N & P	...	120-123°
			O & P	...	180°

The work on the isolation of the corresponding acids of the *ortho*-series, is also complete and these will be described in a subsequent paper. The results obtained suggest that none of those acids is identical with any of the acids hitherto described in the *para*- and *meta*-series. Thus the possibility of the admixtures of different methyl*cyclo*hexanones in the samples of the ketones used as starting materials, have been precluded.

EXPERIMENTAL.

Ethyl 3-methyl*cyclo*hexylidene-cyanoacetate was obtained by the condensation of *m*-methyl*cyclo*hexanone and ethyl cyanoacetate in equimolecular proportion in 76% yield, b. p. 192°/60 mm. or 178-179°/28 mm; $d_4^{30\cdot6}$, 1·01829; $n_D^{30\cdot6}$ 1·48544 $(R_L)_D$ = 58·31, (Calc.: C, 56·36, Exaltation is due to the conjugated system, *cf.* Perkin, Hardinge and Haworth, *J. Chem. Soc.*, 1908, **93**, 1958).

3-Methyl*cyclo*hexane-1-carboxy-1-acetic acid was prepared by the condensation of ethyl 3-methyl*cyclo*hexylidene-cyanoacetate (100 g.) with potassium cyanide (60 g.) dissolved in rectified spirit (430 c. c.). The

alcohol was removed under reduced pressure from a water-bath. The residue, which consisted of the potassium compound of the condensation product with some unchanged potassium cyanide was acidified with concentrated hydrochloric acid (700 c. c.) and then heated on a sand bath for 36 hours. The acid solution when cooled, gave on extraction with ether a thick viscous mass. This was freed from any neutral product by dissolving in a solution of sodium carbonate, which yielded a mixture of acids weighing 100 g. The mixture of acids was separated as follows:

The mixture of acids was digested with ammonia till a distinct smell of ammonia was observed. The product was evaporated to dryness on a water-bath and the residual viscous mass was treated with just enough hot absolute alcohol (400 c. c.). The solution on being cooled deposited crystals of ammonium salt which were filtered off and the solvent removed by distillation. The aqueous solution of the solid ammonium salt yielded on acidification an oily substance. The oily acid was extracted with ether and after removal of the solvent the residue gradually solidified in a vacuum desiccator. The solid thus obtained was crystallised from benzene, when the m. p. began to rise gradually. After nine crystallisations no further rise in the m. p. was noticed. The acid, however, crystallises out along with some benzene and true m. p. cannot be obtained until it has been left to dry for some time. The combination is a loose one and on leaving it for some time at the ordinary temperature, the benzene goes away completely. The sample for analysis was obtained after twelve crystallisations when it came out in small cubes melting sharply at 99°. (Found: C, 59·9; H, 8·3; M. W. by titration, 200. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·0 per cent. M. W., 200).

The *anhydride* of this acid was obtained by heating it with acetic anhydride, b. p. 152-53°/10 mm. It solidified into beautiful rhombic cubes, m. p. 45°, in a vacuum desiccator. When aniline and this anhydride were mixed together in equimolecular proportion and shaken well the *anilic acid* crystallised out readily. This was purified through its sodium salt and recrystallised from rectified spirit in hexagonal laminæ, m. p. 170°. (Found: C, 70·0; H, 8·0. $C_{16}H_{21}O_3N$ requires C, 69·8; H, 7·6 per cent). The *anil*, obtained from this anilic acid, crystallised from dilute alcohol, m. p. 115° (Found: C, 74·7; H, 7·4. $C_{16}H_{19}O_2N$ requires C, 74·7; H, 7·4 per cent).

p-Toluidinamic acid, obtained from the anhydride and *p*-toluidine, crystallises from rectified spirit, m. p. 172°. (Found: C, 70·3; H, 7·9. $C_{17}H_{23}O_3N$ requires C, 70·6; H, 7·9 per cent). When *p*-toluidinamic acid was heated at 190° it decomposed and gave the corresponding *imide* which after being washed with a solution of sodium carbonate, crystallised from

alcohol, in small rhombic plates, m. p. 98°. (Found: C, 75·3; H, 7·9. $C_{17}H_{21}O_2N$ requires C, 75·3; H, 7·8 per cent).

β-Naphthylamic acid, obtained from the same anhydride in the usual way, crystallised from rectified spirit, m. p. 182°. (Found: C, 73·7; H, 7·2. $C_{20}H_{23}O_3N$ requires C, 73·8; H, 7·0 per cent). *β-Naphthylimide* crystallised from dilute alcohol, m. p. 118°. (Found: C, 78·9; H, 6·5. $C_{20}H_{21}O_2N$ requires C, 78·4; H, 6·8 per cent).

The *imide* of the acid was obtained by heating the ammonium salt at the ordinary pressure when it decomposed and gave a solid product which was insoluble in water. This was then washed with water and crystallised from rectified spirit, m. p. 91°. (Found: C, 66·1; H, 8·3. $C_{10}H_{15}O_2N$ requires C, 66·3; H, 8·3 per cent).

From the benzene mother-liquor of the acid (B), the solvent was removed and the residue was crystallised again from benzene. The mother-liquor contained a low melting acid which was obtained as a crystalline solid, m. p. 88° (Found: C, 59·9; H, 7·9 per cent. M. W. 203). This acid (A) is much more soluble in benzene than the acid (B). It does not ordinarily come out from excess of this solvent. It can easily be converted into the anhydride which was obtained as a liquid at the ordinary temperature, b. p. 207°/35 mm, and gave an *anilic acid* which separated out from the benzene solution of the anhydride and aniline. It crystallises from rectified spirit in rhombic plates, m.p. 166° (Found: C, 69·9; H, 7·8 per cent). The corresponding *anil* was prepared by decomposing the anilic acid at 180° and it crystallised from alcohol in prismatic needles and melted at 100° (Found: C, 74·8; H, 7·4 per cent). p-*Totuidinamic acid* separated in large plates from alcohol, m. p. at 172° (Found: C, 71·02; H, 7·9 per cent). The *p-toluidinimide* crystallised from dilute alcohol, m. p. at 78° (Found: C, 75·7; H, 7·9 per cent). The *imide* was prepared by the decomposition of the ammoninm salt. It was found to be rather soluble in rectified spirit, but crystallised out from dilute alcohol in small cubes, m. p. 138·39°. (Found: C, 66·7; H, 8·4 per cent).

The portion of the ammonium salt that remained in alcoholic solution was obtained as a viscous mass on the removal of the solvent. This was then treated with cold water, when most of it dissolved leaving a small quantity which on crystallisation from dilute spirit melted indefinitely between 138-144°. The aqueous solution on acidification gave a mixture of the acids which on being treated with hot benzene left a crystalline insoluble solid, while the oily portion dissolved in it completely. The benzene mother-liquor on being cooled and treated with petroleum (b.p. 70-80°) gave some more of the same solid acid. This acid could be crystallised from ethyl

acetate in large hexagonal prisms, m.p. 168° [Found: C, 59·8; H, 8·1 per cent. M. W. by (titration) 200]. When this acid (C) (5·9 g.) was treated with acetic anhydride (7 c.c.) and the product distilled, the resulting *anhydride* boiled at 159°/9 mm. and gave an *anilic acid* crystallising from dilute alcohol in rhombic plates, m.p. 173° (Found: C, 69·8; H, 7·7 per cent). The anilic acid decomposed at 180-190° and yielded the *anil* which crystallised from spirit in long prisms, m.p. 136° (Found: C, 74·6; H, 7·3 per cent). p-*Toluidin amic acid* crystallised from spirit in rhombic plates, m.p. 182° (Found: C, 70·5; H, 8·3 per cent). The corresponding p-*toluidinimide* came out from alcohol in well defined long prisms, m.p. 141° (Found: C, 75·7; H, 7·9 per cent). *β-Naphthylamid acid* was prepared in the usual way and crystallises from dilute alcohol, m.p. 191° (Found: C, 78·6; H, 6·4 per cent). The *imide* corresponding to this naphthylamic acid crystallises in small cubes from alcohol, m.p. 189° (Found: C, 77·9; H, 6·6 per cent). The *imide* obtained from the ammonium salt of this acid (C) crystallised from spirit in small cubes, m.p. 183° (Found: C, 66·7: H, 8·3 per cent).

The benzene-petroleum mother-liquor from the acid (D) was heated on the water-bath to distil off the solvents. The residue did not solidify even on being left for several months in a vacuum desiccator. It was then esterified by heating on the water-bath with five times its weight of absolute alcohol and 5 % sulphuric acid. The nature of the product of esterification depended upon the duration of heating. When the mixture was heated for 5 hours, the product consisted of an acid portion, which was converted into the calcium salt and found on analysis to be the calcium salt of the acid ester of the succinic acid (II). Hydrolysis of this acid ester with hydrochloric acid (15%), gave acid (B) as the only product. The neutral portion consisted of an ester, b.p. 153°/17 mm; d_4^{30}, 1·03682 n_D^{30}, 1.45677 whence $[R_L]_D$ found, 67·3, calc., 67 9. This ester on being hydrolysed with hydrochloric acid gave a mixture of acids from which benzene precipitated a crystalline solid, which recrystallised from ethyl acetate and melted at 168° and was found to be identical with acid (C). The benzene mother-liquor on evaporation of the solvent deposited a crystalline solid, which could be crystallised from a small qaantity of the solvent in small plates, m.p. 157°. (Found: C, 59·7; H, 7·8 per cent. M., W., 203). When boiled with acetic anhydride it gave an *anhydride* which distilled at 232°/55 mm. and solidified on being left in a vacuum desiccator, m.p. 66°. When the anhydride was mixed in benzene solution with aniline at first no precipitate was obtained. On keeping for some time and shaking, a very small quantity of a crystalline solid separated out. The benzene mother-liquor was, therefore, heated on the water-bath till the solvent was completely removed and a

crystalline residue was obtained. The *anilic acid* thus prepared was purified in the usual way when it came out in large plates from dilute alcohol, m.p. 171°. (Found: C, 70·4; H, 7·9 per cent). The *imide* of the acid was prepared as in the previous cases. It crystallised from rectified spirit in small cubes, m.p. 176-77°. (Found: C, 66·6; H, 8·5 per cent).

One of us (A. T. M.) expresses his gratitude to the Director of Public Instruction, Bengal, for the award of a research scholarship, during the tenure of which this work was completed.

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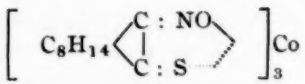
A METHOD FOR THE ESTIMATION OF COBALT IN PRESENCE OF NICKEL.

By Dines Chandra Sen.

The solubility of the greenish yellow precipitate of nickel *iso*nitrosothiocamphor in dilute hydrochloric acid solution and the insolubility of the cobalt complex in the same solvent have been utilised in estimating cobalt in presence of nickel.

The possibility of the use of *di-iso*nitrosothiocamphor as an indicator in alkalimetry has already been reported (Sen, *J. Indian Chem. Soc.*, 1935, **12**, 752). *l-iso*Nitrosothiocamphor, prepared from *l*-thiocamphor, can also be used for the same purpose and the p_H range in both cases has been found to be between 8·6 – 9. In this connexion the author studied the effects of various metallic salts on 1% alcoholic *iso*nitrosothiocamphors. An ammoniacal solution of the reagent was found to be very sensitive in the case of copper and cobalt giving brown and red colouration with sensitiveness of 1: 450,000 and 1 : 50,000 respectively. The reagent was found to be without any effect on salts of iron, aluminium, chromium, zinc, magnesium, manganese and barium.

The solubility of the greenish yellow precipitate of nickel *iso*nitrosothiocamphor in dilute hydrochloric acid and the insolubility of the cobalt complex in the same solvent has been utilised for the quantitative estimation of cobalt in presence of nickel. The cobalt salt has been found to possess the formula $C_{30}H_{42}O_3N_3S_3Co$, and is represented as



Experimental.

For the purpose of estimating cobalt, when present alone, the solution containing not more than 0·01 g. – 0·04 g. of cobalt was diluted to 200 c.c. with water and heated to boiling. 50 C.c. of 10% sodium acetate solution were added followed by an 1% alcoholic solution of *iso*-nitrosothiocamphor till there was no further precipitation. The mixture was thoroughly stirred and the scarlet-red precipitate was filtered through a a sintered glass crucible, washed with hot water to remove sodium acetate, then with *N*/5-NaOH to remove any excess of the reagent. It was then

washed with 2*N*-HCl and finally once again with hot water. It was then dried at 105°-110° and weighed.

The precipitate can also be collected on a filter paper, washed with hot water, ignited and finally weighed as cobalt sulphate.

In presence of nickel, the same procedure was followed except that 30 c.c. of 2*N*-HCl were added after the precipitation and the mixture allowed to stand for 30-40 minutes at room temperature before filtering.

Ni taken.	Co taken.	Wt. of cobaltic complex found.	Weight of *iso*nitroso-thiocampher method (I)	Cobalt found by Ignition to $CoSO_4$.
–	0·02059 g.	0·2252 g.	0·02054g.	0·2052 g
–	0·02059	0·2261	0·02062	0·2063
–	0·02059	0·2256	0·02057	–
–	0·0103	0·1133	0·01033	0·1032
–	0·0103	0·1127	0·01028	0·1032
–	0·0103	0·1125	0·01026	–
0·00993 g.	0·0206	0·2262	0·02063	–
0·01985	0·0206	0·2264	0·02065	0·02067
0·0397	0·0206	0·2256	0·02057	–
0·00993	0·0103	0·1127	0·01028	0·01036
0·01985	0·0103	0·1134	0·01032	0·01039

A solution of chemically pure salt of known concentration was used as the standard.

Discussion.

The present reagent has got certain advantages over others. α-Nitroso β-naphthol (Ilinsky and Knorre, *Ber.*, 1885, **18**, 699; Knorre, *Z. angew. Chem.*, 1893, **26**, 246) being sensitive to light cannot be preserved for a long time and it is difficult to obtain a directly weighable precipitate with this. Rubeanic acid (Ray and Ray, *J. Indian Chem. Soc.*, 1926, **3**, 118) also does not give a directly weighable precipitate of cobalt and does not permit of its separation from nickel. The molecular weight of the *iso*nitrosothiocamphor cobalt complex is very high giving about 9% Co.

Experiments are in progress for the estimation of cobalt by colorimetric and micro methods with the help of this reagent.

My sincere thanks are due to Dr. P. K. Bose for his kind interest in this investigation and also for the facilities of his laboratory.

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USE OF VANADOUS SULPHATE AS A REDUCING AGENT. PART III. ESTIMATION OF CERIUM.

By Pares Chandra Banerjee.

Cerium has been estimated volumetrically in the presence of other rare earths (*e.g.*, in monazite sand) by reducing it with vanadous sulphate. Cerous salt is first oxidised to the ceric state with ammonium persulphate in presence of a measured amount of sulphuric acid. After removing the excess of persulphate the ceric solution is titrated with vanadous sulphate using diphenylamine as an internal indicator.

Cerium and its compounds are at present very important commercial products and a number of methods (Knorre, *Z. anorg. Chem.*, 1897, **11**, 685, 717; Power and Shedden, *J. Soc. Chem. Ind.*, 1900, **19**, 636; Brauner, *Chem. News*, 1895, **71**, 285; Willard and Young, *J. Amer. Chem. Soc.*, 1928, **50**, 1379) has been proposed for its accurate determination. In the gravimetric procedures, the separation of cerium from the other rare earths when occuring together is absolutely essential—a process which is very tedious and at the same time consumes too much time. Volumetric procedures are, however, much simpler. It depends either upon the oxidation of Ce^{+++} to Ce^{++++} or reduction of Ce^{++++} to Ce^{+++}. It is much more rapid and no separation is necessary from the other rare earths. Among the volumetric processes Knorre's classical method of reducing the ceric salt with an excess of hydrogen peroxide and titrating the excess with potassium permanganate is mostly employed for the estimation of cerium. Cerous salt is first oxidised to the ceric state by an alkali persulphate. The exact conditions for carrying out the oxidation have been carefully studied by Knorre (*loc. cit.*) and also by Lindeman and Hafsted. (*Z. anal. Chem.*, 1927, **70**, 4:3).

In a previous communication (Banerjee, *J. Indian Chem. Soc.*, 1935, **12**, 198), it has been stated that ceric salts in acid solution are reduced by vanadous sulphate.

A method based on this reaction has been developed for the quantitative estimation of cerium. The ceric salt is reduced with an excess of vanadous sulphate which is then titrated back with a standard potassium permanganate solution; or the ceric sulphate is directly titrated with the vanadous sulphate solution using diphenylamine as an internal indicator. The end-point is quite sharp and is indicated by the disappearance of the beautiful bluish purple colour which is produced by the interaction of ceric ion with diphenylamine. Experiments were carried

out using both the methods and the results were found to be satisfactory. In the presence of iron, selective reduction takes place and it is only when the blue colour has completely disappeared that the iron begins to be reduced. When the ceric salt is reduced with an excess of vanadous sulphate as stated above, the iron is no doubt reduced to the ferrous state, but it is completely reoxidised at the time of back titration with potassium permanganate.

The cerium solution must be absolutely free from nitric acid or nitrates or any other substances which may oxidise the vanadous sulphate solution.

EXPERIMENTAL.

Analysis of Pure Cerous Oxalate.

Pure cerous oxalate, $Ce_2(C_2O_4)_3$, $10H_2O$ (4·7766 g.) free from other rare earths was heated in a porcelain basin with concentrated sulphuric acid until white fumes of sulphur trioxide were evolved. After cooling, the mass was diluted with water and the solution made up to one litre. A known volume of this solution was oxidised by ammonium persulphate, strictly following the conditions as laid down by Knorre (*loc. cit.*) and Lindemann and Hafsted (*loc. cit.*), cooled to the room temperature and diluted to about 200 c.c. with air-free distilled water. 5-10 C.c. of concentrated sulphuric acid and two drops of an 1% solution of diphenylamine in sulphuric acid were added and the solutions titrated with the vanadous sulphate in an atmosphere of carbon dioxide until the blue colour had completely disappeared. The strength of the vanadous sulphate solution was determined with a standard ceric sulphate solution.

TABLE I.

Strength of vanadous sulphate soln. = 1·092 $N/10$, 1 c.c. = 0·01531 g. of Ce.

No.	Amount of Ce-oxalate taken.	Vol. of vanadous soln. reqd.	% of Ce found.	% of Ce in $Ce_2(C_2O_4)_3$, $10H_2O$.
I.	100 c.c. (0·4776 g.)	12 c.c.	38·48%	38·71%
II.	100 (,,)	11·95	38·32	
III.	125 (0·5970)	15·0	38·47	
IV.	150 (0·7164)	18·05	38·59	

In the second series of estimations ceric salt was reduced with an excess of vanadous sulphate solution and the excess was titrated back with 0·1*N*-potassium permanganate solution. Vanadous sulphate was in this case standardised with permanganate.

TABLE II.

Strength of VSO_4 soln. = 1·78 *N*/10.

No.	Amount of Ce-oxalate taken.	Vol. of vanadous soln. added.	Vol. of $KMnO_4$ soln. added.	% of Ce found.	% of Ce in $Ce_2(C_2O_4)_3$, $10H_2O$.
I.	50 c.c. (0·2388 g.)	10 c.c.	11·25 c.c.	38·45%	
II.	75 (0·3582)	15	16·9	38·37	38·71%
III.	100 (0·4776)	20·0	22·55	38·31	
IV.	100 („)	20·1	22·7	38·39	

Analysis of a Commercial Sample of Ceric Sulphate.

A solution of about 0·1*N* strength was prepared by dissolving the ceric sulphate in water containing sufficient sulphuric acid so as to prevent hydrolysis. A known volume of this solution was titrated in the usual way with vanadous sulphate and the results were compared with the values obtained by Knorre's method (*loc. cit.*).

TABLE III.

Strength of vanadous sulphate soln. = 1·227*N*/10.
1 c.c. = 0·01721 g. of Ce.

No.	Vol. of ceric sulphate soln.	Vol. of vanadous soln. reqd.	Amount of Ce found by present method.	Knorre's method.
I.	10 c.c.	7·20	0·01239 g./c.c.	
II.	20	14·45	0·01243	0·01248 g./c.c.
III.	25	18·05	0·01242	
IV.	25	18·0	0·01239	

Estimation of Cerium in Monazite Sand.

The sand (11·3562 g.) was digested with concentrated sulphuric acid and after filtering and washing the insoluble residue the solution was made up to 1 litre. From a known volume of this solution, the rare earths after precipitation as oxalate were dissolved in sulphuric acid and the cerium was estimated in the same way as described in the analysis of pure cerous oxalate.

TABLE IV.

Strength of vanadous soln. = 1·092N/10.
1 c.c. = 0·01531 g. of Ce.

No.	Wt. of monazite sand taken.	Vol. of vanadous soln. used.	Percentage Ce found by Author.	Knorre's method.
I.	0·4542 g. (40 c.c.)	7·0 c.c.	23·59%	
II.	0·5678 (50)	8·70	23·47	23·6%
III.	0·9084 (80)	14·1	23·76	

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VISCOSITY AND DENSITY OF CADMIUM CHLORIDE SOLUTIONS AT 35°.

By Amritansu Sekhar Chakravarti and Balbhadra Prasad.

Viscosity and density of solutions of $CdCl_2$ both in water and hydrochloric acid (0·100 *N*) have been measured. Jones and Dole equation is applicable to both the solutions. The value of '*A*' as found from aqueous solutions is 0·013 and 0·0079 for the hydrochioric acid solutions (for concentrations expressed in g. equivalents) as against 0·0099 calculated from Falkenhagen-Vernon equation for aqueous solutions. The values of '*B*' are 0·131 for water and 0·117 for acid solutions. The applicability of Jones and Dole equation to hydrochloric acid solutions is surprising. It was expected that the electrical field in the solution due to the presence of other ions would make the equation inapplicable. The density of both the solutions increases linearly with concentration to an accuracy of 0·0001 for the dilute range.

The viscosity of aqueous solutions of cadmium chloride was studied, first to see whether it obeyed Jones and Dole equation (Jones and Dole, *J. Amer. Chem. Soc.*, 1929, **51**, 2950),

$$\eta/\eta_0 = 1 + A\sqrt{c} + Bc$$

in the dilute range ; and if it did, whether the value of '*A*' was of the expected order (Falkenhagen and Vernon, *Phil. Mag.*, 1932, **14**, 537). Some concentrated solutions too were examined. Some experiments were made with cadmium chloride dissolved in 0·100 *N*-hydrochloric acid as well.

Experimental.

The experimental procedure followed as well as the dimensions of the pyknometer and viscometer were the same as described in a previous paper (*J. Indian Chem. Soc.*, 1938, **15**, 301). As before, no kinetic and surface tension corrections were necessary. Five readings were taken for each solution. They seldom differed from one another by more than 0·2 second. Two readings for the pyknometer were taken and they always differed by less than 0·001 g. Double distilled water was used in making all the solutions.

The maximum possible error in the value of viscosity η/η_0 as found by assuming a maximum error of 0·2 second in time of transpiration and

0·001 g. in the weight of pyknometer comes out to be ± 0·0003, but the actual error in most cases is expected to be much less.

In Table I concentration (c) of cadmium chloride in g. equivalents per litre, density (ρ) in g./c.c. and relative viscosity (η/η_0), hereafter called viscosity, are given.

TABLE I.

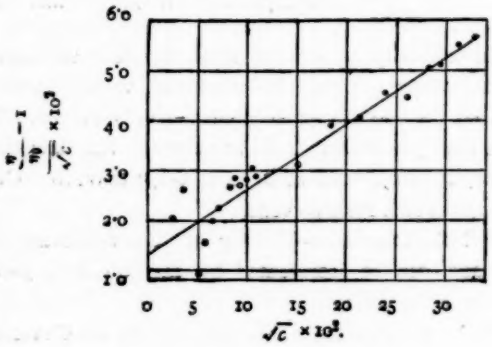
$c \times 10^4$.	ρ.	η/η_0		$c \times 10^4$.	ρ.	η/η_0	
		obs.	calc.			obs.	calc.
0	0·9941	1·0000	1·0000	456·0	0·9977	1·0087	1·0088
6·00	0·9941	1·0005	1·0004	570·0	0·9987	1·0109	1·0106
12·00	0·9941	1·0009	1·0006	684·0	0·9993	1·0117	1·0124
26·00	0·9943	1·0005	1·0010	798·0	1·0001	1·0143	1·0142
33·00	0·9943	1·0009	1·0011	864·0	1·0006	1·0150	1·0151
43·00	0·9944	1·0013	1·0015	972·0	1·0014	1·0172	1·0168
51·00	0·9945	1·0016	1·0016	1080	1·0024	1·0086	1·0185
60·00	0·9947	1·0019	1·0018	2140	1·0105	1·0331	1·0345
68·00	0·9948	1·0022	1·0020	3210	1·0184	1·0483	1·0494
77·00	0·9948	1·0025	1·0021	4280	1·0271	1·0637	1·0644
85·00	0·9950	1·0025	1·0023	5350	1·0347	1·0776	...
99·00	0·9948	1·0028	1·0026	6420	1·0428	1·0925	...
110·0	0·9949	1·0032	1·0028	7390	1·0516	1·1080	...
114·0	0·9950	1·0031	1·0029	8440	1·0587	1·1226	...
228·0	0·9959	1·0047	1·0050	9500	1·0675	1·1398	...
342·0	0·9968	1·0072	1·0069	10700	1·0747	1·1532	...

In Table II, the concentration (c) of cadmium chloride in 0·100*N*-hydrochloric acid, density and viscosity are given.

TABLE II.

$c \times 10^4$.	ρ.	η/η_0		$c \times 10^4$.	ρ.	η/η_0	
		obs.	calc.			obs.	calc.
0	0·9958	1·0081	1·0081	90·00	0·9965	1·0094	1·0100
10·00	0·9960	1·0086	1·0085	100·0	0·9966	1·0096	1·0101
20·00	0·9961	1·0089	1·0087	200·0	0·9975	1·0114	1·0115
34·00	0·9961	1·0089	1·0090	300·0	0·9983	1·0135	1·0130
40·00	0·9963	1·0088	1·0091	400·0	0·9990	1·0150	1·0144
50·00	0·9963	1·0089	1·0093	500·0	0·9998	1·0159	1·0158
60·00	0·9963	1·0090	1·0094	600·0	1·0007	1·0167	1·0171
70·00	0·9964	1·0093	1·0096	700·0	1·0014	1·0184	1·0184
80·00	0·9965	1·0097	1·0097	800·0	1·0022	1·0196	1·0198

In the figure $(\eta/\eta_0 - 1/\sqrt{c})$ for aqueous solutions is plotted against $\sqrt{c}$, *i.e.*, against square root of equivalent concentration. A similar figure is obtained in the case of hydrochloric acid solutions also.



DISCUSSION.

Viscosity of Aqueous Solutions.—In the figure a straight line ($A = 0{\cdot}013$ and $B = 0{\cdot}131$) is obtained. The value of 'A' as calculated from Falkenhagen-Vernon equation (*loc. cit.*) by taking the dielectric constant of water to be 74 at 35°, and by using extrapolated values of the ionic conductivities of Cd^{++} and Cl^- given in Landolt-Börnstein tables, is 0·014 when c is expressed in g. mols. and so 0·0099 when c is in g. equivalents, as against 0·013 found by actual experiment. The two values are of the same order. The calculated values of viscosity are given in Table I. The calculated viscosities

for seventeen out of twenty-two solutions (range tenth normal) agree with the observed values within experimental error (± 0.0003). In three, the difference rises upto 0·0004, in one upto 0·0005 and in one upto 0·0007.

Density of Aqueous Solutions.—Density of aqueous solutions increases almost linearly with concentration and can be represented almost within experimental error ($\pm$ 0·0001) upto a concentration of 0·2140 by the equation: $\rho = 0.9941 + 0.0773c$. Beyond this concentration, the variations become wider and are not within the limits of experimental error.

Viscostiy of HCl-$CdCl_2$ *Solutions.*—All the solutions were made in 0·100 *N*-hydrochloric acid solution ($\rho = 0.9958$, $\eta/\eta_0 = 1.0081$). These experiments were performed to see whether hydrochloric acid in the solutions behaved as a mere solvent or exerted such electrical forces on the ions of $CdCl_2$ as to render Jones and Dole equation inapplicable. In this case, $\eta/\eta_0 - 1.0081/\sqrt{c}$ was plotted against $\sqrt{c}$. A straight line ($\eta/\eta_0 = 1.0081 + 0.0080\sqrt{c} + 0.117\,c$ or $\eta/\eta_{HCl} = 1 + 0.0079\sqrt{c} + 0.116c$) was obtained which represents the viscosity of all the solutions quite well, though the difference between the observed and the calculated values in many cases (*vide* Table II) exceeds the possible experimental error(± 0.0003). The only perceptible effect of the hydrochloric acid solution is the lowering of the value of A from 0·013 to 0·0079 and of B from 0·131 to 0·116. Otherwise, hydrochloric acid behaves as a mere solvent.

Density of HCl-$CdCl_2$*Solutions.*—Density of the solutions increases linearly throughout and can be represented by the equation, $\rho = 0.9958 + 0.0807c$ to an accuracy of ± 0.0001g.

One of us (A.S.C.) acknowledges his gratitude to the Government of Orissa in the Ministry of Education for the grant of a scholarship.

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ON THE MOVING BOUNDARY METHOD FOR THE DETERMINATION OF CATAPHORESIS OF COLLOIDS.

By N. C. Sen-Gupta.

A colloid may be looked upon as a solution of mixed electrolytes so that the relation of Kohlrausch and Weber is applicabie in the measurement of cataphoresis by the method of moving boundaries. It has been shown in the following that under proper conditions the results of the moving boundary method are reproducible within two per cent and that the results obtained with boundary method and with microscopic method are identical within the limit of experimental errors.

Three methods are available for measuring cataphoretic speed of colloids namely, (*i*) the moving boundary method, (*ii*) the microscopic method and (*iii*) the titration method.

The first one has a more general applicability. There are, however, some difficulties which seem to have set a limit to the wide acceptance of the moving boundary method. Some of these difficulties have been overcome in the method developed by Mukherjee and co-workers, which lies on a recognition of the conditions at the boundary based on the Kohlrausch-Weber theory of ionic migration (*Proc. Roy. Soc.*, 1923, **A 103**, 102 ; *J. Indian Chem. Soc.*, 1928, **5**, 593 ; *J. Phys. Chem.*, 1932, **36**, 593 ; *Kolloid Z.*, 1933, **62**, 257 ; *cf.* also Freundlich and Zeh, *Z. physikal. Chem.*, 1925, **114**, 84; Kruyt and Willigen, *Kolloid Z.*, 1928, **44**, 11; Hacker, *Koll.-Chem. Beih.*, 1935, **41**, 147).

In the boundary method the colloidal solution comes in contact with a foreign electrolyte which may influence the mobility of the particles in several ways. In order to establish the suitability of the boundary method a comparison of the latter with microscopic method will be valuable, since in the microscopic method no foreign electrolyte is added. A comparison of the moving boundary method with other methods has been made by Kruyt and Willigen (*loc. cit.*), Henry and Brittain (*Trans. Faraday Soc.*, 1933, **29**, 807) and Komagata (*Tokyo Eletro-Tech. Lab. Japan*, 1933, No. 348).

In the present investigation a comparison of the cataphoretic speed of particles of vanadium pentoxide hydrosol by the microscopic and moving boundary methods has been made and a satisfactory agreement has been obtained. In this connection the theory of Kohlrausch and Weber has been developed and verified with certain mixtures of potassium chloride and iodo-eosinate.

The theory of Kohlrausch and Weber holds quantitatively for the migration of a boundary between two single electrolytes having a common ion (Sen-Gupta, *J. Indian Chem. Soc.*, 1937, **14**, 685) and also for mixtures of electrolytes (Hacker, *loc. cit.*). Consider the simple case of a mixed electrolyte containing two binary electrolytes AR and BR having the common ion R. Let C_A , C_B and C_R represent the concentrations and V_A , V_B and V_R the rates of migration of the ions. Let B be the slower moving ion, *i.e.*, $V_A > V_R$. For a boundary between the mixture and AR as shown in Fig. 1, the following relation should be satisfied according to the theory of Kohlrausch and Weber if the composition of the two electrolytes were to remain unchanged during the movement of the boundary between them.

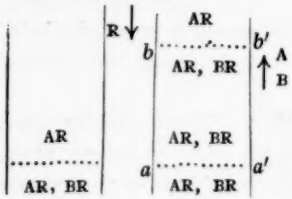

FIG. 1.

$$C'_A = C_A + C_B \frac{V_A (V_B + V_R)}{V_R (V_A + V_R)} \qquad \ldots \quad (i)$$

where C'_A represents the concentration of A ion in the pure electrolyte AR. If the original concentration of AR and BR in the mixture be different from that demanded by the above equation then a more dilute or more concentrated solution will come out of the original mixture and fill the region $aa'bb'$ so that the above relation is satisfied.

It follows from equation (*i*), that

(*a*) if C_A is zero, $C'_A = C_B \frac{V_A (V_B + V_R)}{V_R (V_A + V_R)}$;

(*b*) if C_B is very small, the two solutions become nearly equiconducting and the velocity of the boundary indicates the velocity of B ions only ;

(*c*) for intermediate values of C_A and C_B , one can obtain V_B directly from the speed of the boundary and the potential gradient in the mixed electrolyte layer, but V_A cannot be directly obtained. This case is illustrated by the following measurements.

Measurement with Mixture of Electrolytes.

For a given value of C'_A , C_A and C_B may have any number of values (*vide* Eqn. *i*) A 0·02*N*-potassium chloride was taken as the leading electrolyte and a series of mixtures of potassium chloride and iodoeosinate were prepared so as to satisfy equation (*i*) in every case. The potential gradient in each layer was calculated indirectly from the current strength and the specific conductivity (the diameter of the tube was known from previous measurements, *cf.* Sen-Gupta, *loc. cit.*). The results are given below.

TABLE I.

Ratio of KCl in the mixture and in the leading electrolyte.	(Speed of the boundary) / (Pot. grad. in KCl layer)	(Speed of the boundary) / (Pot. grad. in the mixture)
0	$86{\cdot}3 \times 10^{-5}$ cm./sec.	$34{\cdot}9 \times 10^{-5}$ cm./sec.
0·05	75·7	34·5
0·20	64·2	34·3
0·40	56·0	35·5

With increasing concentrations of KCl in the mixture the restoring force which opposes diffusion becomes relatively weak and the boundary loses its sharpness. It is evident, however, from Table I, that the absolute velocity of the slow-moving indicator ion in a mixed electrolyte can be directly obtained by the method of moving boundaries.

The results of some previous experiments with mixtures of electrolytes from this laboratory (*cf.* Mukherjee, Mitra and Sen-Gupta, *J. Indian Chem. Soc.*, 1936, **13**, 64, Table XIII) may also be interpreted in the light of this theory. A boundary was formed between a mixture containing 0·009*N*-HCl and 0·005*N*-HPic and a pure 0·01*N*-HCl solution. The two solutions were not initially adjusted so that a new solution came out of the mixed electrolyte. The potential gradient in the new mixture was directly observed and the velocity of picrate ion was obtained from the speed of the boundary and the potential gradient as 34·4 ± 1·2 ; this agrees fairly well with the value of V_{Pic}, *i.e.*, 35·4 (*cf.* Sen-Gupta, *loc. cit.*).

A colloid may be looked upon from the point of view of the migration of ions in an electric field as a mixture of electrolytes, the colloidal micelle being regarded as a large polyvalent ion. Measurements were next made with a colloidal solution of vanadium pentoxide whose equivalent concentration is sufficiently small as in case (*b*) above.

EXPERIMENTAL.

A vanadium pentoxide sol of good age, prepared from ammonium vanadate and hydrochloric acid, was taken. The specific conductivity of the sol did not change during the period of measurements. The tube and procedure were the same as used in a previous investigation (Sen-Gupta, *loc. cit.*).

Composition of the Sol.

The sol contained 1·95 g. of V_2O_5 per litre as found by (*a*) evaporating a given amount of the sol and by (*b*) titrating a given amount of the dissolved sol potentiometrically with ferrous sulphate. The p_H of the sol was 3·01 as measured with a glass electrode and a quinhydrone electrode. The

hydrogen ion concentration is thus $9{\cdot}8 \times 10^{-4}N$. The sol was, however, found to react with quinhydrone and the observed p_H changed with time. The chlorine ion concentration was measured with Ag-AgCl electrode (Noyes and Ellis, *J. Amer. Chem. Soc.*, 1917, **39**, 2534) and found to be $9{\cdot}9 \times 10^{-4}N$ assuming the activity to be equal to the concentration. The sol thus contains about $9{\cdot}8 \times 10^{-4}N$-hydrochloric acid, whose specific conductivity at 35° is $49{\cdot}5 \times 10^{-5}$ mho. The specific conductivity of the sol was measured and found to be $50{\cdot}08 \times 10^{-5}$ mho. Hence the contribution of the colloid micelles to the conductivity of the sol is nearly 1%. The specific conductivity of the ultrafiltrate obtained with cellophane membrane is $48{\cdot}18 \times 10^{-5}$ mho, the chlorine ion concentration being $9{\cdot}6 \times 10^{-4}N$; the conductivity of the sol is thus almost entirely due to that of the intermicellary electrolytes, namely $H^{\cdot}$ ion and Cl' ion.

Choice of Leading Electrolyte.

According to equation (*i*), an equiconducting hydrochloric acid solution should be suitable as the leading electrolyte. Measurements have been carried out with different concentrations of hydrochloric acid. The falling boundaries were somewhat diffuse. This is to be expected. Only rising boundaries were taken. In columns 3 and 4 of Table II are given the potential gradients in the leading electrolyte E_1, and the original sol layer E_0 respectively. The fifth column gives the average potential gradients E_2, behind the moving boundary. The average potential gradient is obtained from the relation,

$$E_2 = E_1 + \frac{dE}{dx} \qquad \ldots \quad (ii)$$

where dE/dx is the rate of increase of the potential with the progress of the boundary as measured between two side-limbs enclosing the boundary (*cf.* Mukherjee, Mitra and Bhattacharya, *J. Indian Chem. Soc.*, 1935, **12**, 177).

TABLE II.

Temp. = 35°. Cross section of the tube = 0·333 sq. cm.

Sp. conducty. of HCl × 10^5 mho.	Current amp. × 10^5.	E_1 (volt/cm.).	E_0 (volt/cm). Calc.	E_2 (volt/cm.).
35·06	11·2	0·94	0·65	0·82
40·20	11·5	0·86	0·68	0·80
45·63	19·2	1·27	1·16	1·24
50·67	21·8	1·29	1·29	1·29
55·70	22·4	1·20	1·34	1·30
61·01	24·5	1·22	1·47	1·37
67·27	26·0	1·16	1·56	—

The values of E_2 were, however, not very constant. According to the theoretical considerations outlined above, E_2 ought to have been equal to E_1. The deviation of E_2 from the normal value is to be sought to the mixing process taking place at aa', the initial position of the boundary (*vide* Fig. 1) so that the final value becomes intermediate between E_1 and E_0.

The rate of displacement of the boundary was, however, constant within 1% during the period of measurement for all the cases investigated. The results are given in Table III. The cataphoretic velocity has been calculated from the speed of the boundary and the potential gradient in the hydrochloric acid layer.

TABLE III.

Temp. = 35°. Sp. conductivity of the sol = $50{\cdot}08 \times 10^{-5}$ mho.

Sp. conducty. of the leading elcetrolyte × 10^5 mho.	$C.V.$ × 10^5 cm./sec.	$C.V.$ (mean) × 10^5 cm./sec.
35·16	86·7, 86·3, 87·7, 88·7, 88·7	87·6
40·20	87·1, 85·3, 85·5	86·0
45·63	85·6, 84·8, 84·9	85·1
50·67	85·1, 85·3, 84·8, 84·2	84·9
55·70	85·4, 84·6, 84·5	84·8
61·01	85·9, 85·5, 83·7, 85·4	85·1
67·27	84·5, 84·9, 84·9	84·8

It is thus seen that moving boundary method is capable of giving very accurate results under proper conditions. If, however, hydrochloric acid is substituted by a second electrolyte in the leading solution, the theoretical considerations of Kohlrausch and Weber become inapplicable and the results cannot be expected to be accurate. It was actually observed that with a mixture of KCl and HCl equiconducting with the sol, both the potential gradient E_2 and the rate of displacement of the boundary changed continuously during electrolysis.

Similar observations were previously made on Fe_2O_3 sol (*cf.* Mukherjee, *J. Phys. Chem.*, 1932, **36**, 593). A mixture of HCl and $FeCl_3$ having the same p_H and specific conductivity as that of the sol was found to produce a satisfactory boundary. The sol evidently contained some $Fe^{\cdots}$ ions besides $H^{\cdot}$ and Cl' and the condition that the sol layer will proceed unchanged is that the leading electrolyte must also contain all the electrolytic ions in a fixed proportion. This was found to be the case.

The Moving Boundary Method and the Microscopic Method.

In the microscopic method the cataphoresis was measured in a flat cell at the room temperature (23-24°). The experimental procedure was the same as that used by Mukherjee, Chaudhury and Bhabak (*J. Indian Chem. Soc.*, 1936, **13**, 372). Moving boundary measurements were carried at 25° in a thermostat for comparison.

The true velocity in the micro-cataphoretic method was obtained from the formula,

$$C.V. = \tfrac{3}{4}V_{\frac{1}{6}} + \tfrac{1}{4}V_{\frac{1}{2}} \qquad \ldots \quad (iii)$$

where $V_{\frac{1}{6}}$ and $V_{\frac{1}{2}}$ represent the cataphoretic velocities at 1/6th and half depth of the cell. Since the breadth of the cell was 1 cm. and the thickness only 0·07 cm., the correction for the finite thickness of the cell as suggested by Komagata (*loc. cit.*) is very small for the present case.

TABLE IV.

V_2O_5 *sol.*

Sp. conducty. $= 37{\cdot}0 \times 10^{-5}$ mho at 25°.

Moving boundary method with equiconducting HCl at 25°.		Microscopic method at 23-24°.
$C.V. \times 10^5$.		$C.V. \times 10^{-5}$.
66·6 cm./sec.	66·3 cm./sec.	66·6 cm./sec.
66·8	66·5	66·3
65·8	66·5	66·7
		67·2
Mean 66·4		Mean 66·7

In the microscopic measurements the temperature inside the cell was somewhat higher due to the heating effect of the current. Taking this fact into account the two methods yield results in entire agreement with each other.

My best thanks are due to Prof. J. N. Mukherjee, D.Sc., for his suggesting the problem of the work and kind interest, and to the University of Calcutta for awarding a research scholarship, during the tenure of which this work was carried out.*

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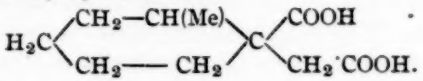
STRAINLESS MONOCYCLIC RINGS. PART III. SYNTHESIS OF 2-METHYL-*CYCLO*HEXANESUCCINIC ACID AND THE SEPARATION OF ITS ISOMERS.

By Muhammad Qudrat-i-Khuda, Akbar Ali Mallick (in part) and Ashutosh Mukherji.

2-Methyl*cyclo*hexane-1-carboxy-1-acetic acid, on being carefully separated, gives four isomeric modifications, melting respectively at 162°, 155°, 153° and 142°. The individuality of the acids has been proved by a study of the properties of their anilic acids, as well as the imides. Of the four acids, two are capable of undergoing change into the other two, through their anhydrides. The same phenomenon has also been observed in the case of the *m*-methyl*cyclo*hexane-succinic acids described in a previous communication. The work as now complete, leaves no doubt as to the validity of the hypothesis of release of strain in *cyclo*hexane ring, under certain conditions.

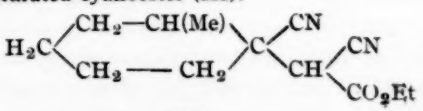
In Parts I and II, it has been shown (*J. Indian Chem. Soc.*, 1931, **8**, 277 ; 1938, **15**, 462) that by introducing substituents inside the *cyclo*hexane ring it is possible to isolate isomeric compounds as required by the multiplaner configurations of the methyl*cyclo*hexane ring. The present communication deals with the isolation of the different isomeric succinic acids (I) from *o*-methyl-*cyclo*hexanone.



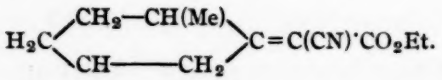
(I)

This acid has been prepared by the same general method, as adopted in the case of its *para-* and *meta*-methyl analogues. The separation of these acids, through their ammoninm salts, has not been possible as in the previous cases, most of the acids undergoing conversion into a mixture of their imides in an attempt to convert them into the ammonium salts.

As the result of a series of very careful experiments it has been found that they could be separated satisfactorily with the help of solvents. A mixture of acids (I) was obtained by the hydrolysis of ethyl 1-methyl*cyclo*hexane-2-cyano-2-cyanoacetate (II), obtained by the addition of potassium cyanide to the unsaturated cyanoester (III).



(II)



(III)

The product of hydrolysis, on treatment with benzene, gave an acid (M), which when carefully crystallised melted at 162°. From the mother-liquor, two acids, one (P), m. p. 142° and the other (O), m. p. 153°, were obtained. All the three acids crystallised from ethyl acetate. When the acid (O) was converted into its anhydride, a liquid, b p., 142°/8 mm, was obtained (the boiling point of the anhydride of P was 160-62°/8 mm.). But when the anhydride of (O) was decomposed by water, the only product was the acid (P), m. p. 142°. The same anhydride yielded an anilic acid identical with that of (P), m. p. 140°.

A very careful search was made for the fourth acid in the products of hydrolysis of ethyl 2-methyl*cyclo*hexane-1-cyano-1-cyanoacetate (II), but to no purpose. The inter-conversion of the two acids (P) and (O) opened up the question whether the acid (M) was also capable of undergoing change into another acid. The anhydride of the acid (M), b p. 202°/24 mm., on hydrolysis gave an acid, m. p. 155°, and not 162°. That this lowering is not due to the presence of any impurity, has been proved. Neither does it appear to be identical with the acid (P), because there is a distinct lowering in its m. p. when mixed with this other acid.

A comparative study of the mixed m. p. of the different acids has been made and the results are given below in Table I, which shows definitely that all the four acids are actually different from one another.

TABLE I.

M. p. of acids.		Mixed m. p. of acids.	
M	162°	M & P	148-49°
N	155°	M & N	149-50°
O	153°	M & O	144°
P	142°	N & O	144°
		N & P	140°
		O & P	139°

Other derivatives of the acids, such as the anilic acids and the imides have also been made and purified very carefully for the determination of their individual melting points, as also their mixed melting points. The results are tabulated below in Tables II and III. These also suggest that the acids are distinctly separate entities. The m. p. of the acid (M) (162°), obtained from *o*-methyl*cyclo*hexanone, although very close to the melting point of the acid (C) from *m*-methyl*cyclo*hexanone, their difference has been demonstrated by the lowering of the m. p. to 147°. Similar difference is also shown by their derivatives.

TABLE II.

M. p. of the anilic acids of			Mixed m. p. of	
M	(W)	150°	W & X	143°
N	(X)	143°	W & Y	138°
O	(Y)	140°	W & Z	139°
P	(Z)	140°	W & Y	137°
			X & Z	135°
			Y & Z	139-140°

TABLE III.

M. p. of imides of			Mixed M. p. of	
M	R	107°	R & S	105°
N	S	110-111°	R & T	97-98°
O	T	98°	R & U	103-4°
P	U	105°	S & T	95-96°
			S & U	105-6°
			T & U	97-98°

Work on the intermolecular changes of the acids from 4-methyl-*cyclo*hexanone is now in progress.

EXPERIMENTAL.

Ethyl 1-*methyl*cyclo*hexane*-1-*cyanoacetate* was prepared in the usual way but the yield of the product never exceeded 50% of the theory, after repeating the condensation several times with *o*-methyl*cyclo*hexanone and cyanoacetic ester, in presence of small amounts of piperidine. On redistillation, the unsaturated ester boiled at 165-67°/41 mm. and had $d_4^{28\cdot8}$, 1·0248, $n_D^{28\cdot8}$, 1·481736 whence $[R_L]_D$ found, 57·54 and calculated, 56·36. (Found: C, 69·2; H, 7·99. $C_{12}H_{17}O_2N$ requires C, 69·5; H, 8·2 per cent).

The unsaturated cyano ester (100 g.) was condensed with potassium cyanide (60 g.) in the usual way, the yield of the acid being satisfactory, only when the condensation mixture was allowed to stand for at least 2 weeks. The hydrolysis of the dicyano ester also took a long time. Not less than 48-50 hours' heating on an wire-gauze would hydrolyse it fairly completely. From the above quantities the mixture of acids obtained amounted to 70 g. only. The acid was purified by dissolving it in a solution of sodium carbonate, from which on acidification the major quantities of the acids were precipitated out. The mother-liquor on evaporation to dryness on a water-bath, and extraction with ether yielded the acid (O).

Separation of the Acids.

The crude mixture of the acids was heated with benzene on a water-bath for 45 minutes with a reflux condenser each g. of the acid mixture requiring 5 c.c. of benzene. It was then filtered hot and the solid acid that remained was washed with cold benzene (about 100 c.c.). The residue consisted of the pure acid (M), m.p. 162° after crystallisation from ethyl acetate. [Found: C, 59·6 ; H, 7·8, M. W. (by titration), 202. $C_{10}H_{16}O_4$ requires C, 60·0 ; H, 8·0 per cent. M.W., 200).

The *anhydride* of the acid (M) was obtained by heating the acid (10 g.) with acetic anhydride (25 c.c.) and acetyl chloride (2 c.c.) for 6 hours on a sand-bath. After the reaction was over, the fraction distilling at 202°/24 mm. was collected.

The *anilic acid*, obtained from the anhydride, crystallises from dilute alcohol, m.p. 150°. (Found: C, 70·1; H, 7·94. $C_{16}H_{21}O_3N$ requires C, 69·8; H, 7·6 per cent).

The p-*toluidinamic acid* was obtained from the anhydride and crystallised from ethyl acetate, m.p. 179°. (Found: C, 70·4; H, 8·0. $C_{17}H_{\ 3}O_3N$ requires C, 70·6; H, 7·9 per cent).

The p-*toluidinimide* was obtained from the corresponding toluidinamic acid on heating the latter in a sulphuric acid bath at 185-95° for 25 minutes. It crystallises from alcohol, m.p. 140°. (Found: C, 75·63; H, 8·0. $C_{17}H_{21}O_2N$ requires C, 75·3 ; H, 7·8 per cent). *β-Naphthylamic acid*, obtained from the anhydride, crystallises from ethyl acetate, m.p. 163°. (Found: C, 73·6; H, 6·8. $C_{20}H_{23}O_3N$ requires, C, 73·8; H, 7·0 per cent).

The corresponding *β-naphthylimide* crystallises from alcohol, m.p. 169°. (Found: C, 78·6 ; H, 7·0. $C_{20}H_{21}O_2N$ requires C, 78·4 ; H, 6·8 per cent).

The *imide* of the acid (M) was obtained by heating the ammonium salt of the pure acid. When the decomposition was complete, the mass was washed with water and crystallised from dilute spirit. It was obtained as shining flakes, m.p. 107°. (Found: C, 66·7 ; H, 8·0. $C_{10}H_{15}O_2N$ requires C, 66·3 ; H, 8·3 per cent).

Pure anhydride of the acid (M) (1·2 g.) was heated with water on a water-bath for 8 hours. A solid acid (N) gradually speparated out, which after being crystallised from water and then from ethyl acetate melted at 155°. (Found: C, 60·2; H, 7·9 per cent). It was converted into the *anhydride* when a liquid anhydride, b.p. 157°/12 mm., was obtained, which when decomposed with water gave the same acid, m.p. 155°. The anhydride yilded an *anilic acid* which crystallised from dilute spirit, m.p. 143°. (Found:

C, 69·9 ; H, 7·5 per cent). The anilic acid is apparently different from the anilic acid previously described and a mixed m. p. determination with equal portions of the two anilic acids showed a definite depression to 143°. The *imide* of the acid was prepared through the ammonium salt and crystallised from hot water, containing a small quantity of alcohol, m.p. 110-11°. (Found: C, 66·5; H, 8·2 per cent).

Acids (P) *and* (O).

From the benzene mother-liquor after the separation of the acid (M), the solvent was removed on a water-bath and the residual semi-solid mass allowed to stand over a week, when a small quantity of the solid separated out. It was then triturated with small amount of cold benzene and filtered. The acid was found to be the same acid, m.p. 162°. From the filtrate, the solvent was removed once again and the residue was esterified in the usual way. It was found, however, that heating the alcoholic mixture on a water-bath for 6 hours was necessary in order to effect a better separation. The product was isolated in the usual way, and it could be separated into two portions, one acidic and the other neutral. The neutral portion consisted mainly of an ester, b. p. 147-48°/18 mm; and gave $d_4^{26\cdot3°}$, 1·03164, $n_D^{26\cdot3°}$, 1·45737 whence $[R_L]_D$ found 67·63; calc., 67·90. (Found: C, 65·4; H, 9·2. $C_{14}H_{24}O_4$ requires C, 65·6; H, 9·3 per cent).

The *ester* was next hydrolysed by means of 15% hydrochloric acid by heating for 18 hours on a sand-bath to an acid, m. p. 151°, which on being crystallised from ethyl acetate melted at 153°. This has been called acid (P). (Found: C, 59·8; H, 8·3 per cent).

The *anhydride* of the acid (P) boiled at 142°/8 mm. It gave *an anilic acid*, which crystallised from dilute alcohol, m.p. 140°. (Found: C, 70·2; H, 7·6 per cent). The *anil* crystallised from very dilute alcohol, m. p. 105°. (Found: C, 74·7; H, 7·3 per cent). *p-Toluidinamic acid* separated in needles from alcohol, m. p. 187°. (Found: C, 70·6; H, 7·9 per cent). *p-Toluidin-imide* crystallised from alcohol in needles, m.p. 130°. (Found: C, 75·1; H, 7·5 per cent). The *imide* of the acid obtained from the ammonium salt of the pure acid crystallised from dilute alcohol, m. p. 98°. (Found: C, 66·4; H, 8·4 per cent). The acid was regenerated from the anhydride when it melted at 141° and not at 153° which is the m. p. of the original acid.

The acidic portion of the product of esterification consisted of an acid ester. It was obtained as a thick viscid mass, but the silver salt was

obtained as a solid product. (Found : Ag 31·9. $C_{12}H_{19}O_4Ag$ requires Ag, 32·2 per cent). It was found, however, that if the esterification process was continued for more than 6 hours, some of the second acid was also converted into the neutral ester and the product of hydrolysis of the neutral ester then yielded a mixture of the two acids one melting at 153° and the other at 142°. When the acid ester previously obtained was hydrolysed with 15% hydrochloric acid by heating on the sand-bath for the same period as the neutral ester, on cooling, a mass of crystalline solid separated which when crystallised from ethyl acetate melted at 142°. This has been called acid (O). (Found: C, 59·8; H, 7·8 per cent). The *anhydride* of the acid, prepared as usual, boiled at 160-62°/8 mm. This, however, was found to give the same anilic acid, m.p. 140° and an anil, m. p. 105°, as the other acid (P). The *p-toluidinamic acid* also melted at 187°, thus showing that the anhydride actually gives the same derivatives as the other anhydride. The regeneration of the acid from the anhydride gave the same acid, m. p. 142°.

The *imide* of the acid, prepared from the pure acid, was found to be a different one. It crystallised from dilute alcohol, m. p. 105°. (Found : C, 66·7; H, 8·4 per cent).

Grateful thanks of one of us (A. T. M.) are due to the Director of Public Instruction, Bengal, for the award of a research scholarship, which has enabled him to carry on this work.

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STUDIES IN ORGANO-ARSENIC COMPOUNDS. PART VIII. SYNTHESIS OF ARSINDOLE DERIVATIVES FROM PHENYLACETYLENE.

By Hirendra Nath Das-Gupta.

The adducts of the primary aryl arsenious halides have been prepared. In the formation of the adducts arsenic attaches itself to the α-carbon atom of the unsaturated grouping with simultaneous ring-closure.

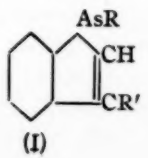
Fischer and Klemperer (*Der Therapie der Gegenwart*, 1-3, Jan. 1913) studied the action of arsenic trichloride on aliphatic unsaturated compounds. Fischer (*Annalen*, 1914, **403**, 106) investigated its action on behenolic acid, but the structure of the chloroarsenobehenolic acid was not actually determined, though there was hardly any doubt about the addition at the triple bond. Mannich (*Arch. Pharm.*, 1935, **273**, 275) reacted it with 1-phenyl-1 : 3-diethylaminopropine ($PhC\equiv C\cdot CH_2\cdot NEt_2$) with the idea of getting an analogous compound. But the reaction failed to give a simple adduct. One of the products was found to be an arsenical ring compound analogous to indole. The formation of the latter involved an unstable addition product of the propine base with arsenic trichloride.

The work of Mochnac and Bagniuk (*Compt. rend.*, URSS., 1937, **14**, 535) on the action of arsenic trichloride on behenolic acid and stearolic acid on the one hand and tetrolic or ethylpropiolic acid on the other, shows the influence of temperature on the nature of the resultant product.

In the present paper adducts have been formed with primary aryl arsenious halide. The addition takes place in such a way that the arsenic attaches itself to the α-carbon of the unsaturated grouping and simultaneous ring-closure occurs.

Phenylacetylene and phenyldichloroarsine reacted with evolution of hydrogen chloride at 120°. The resultant product was found to be an arsenical ring compound in which the ring formation had taken place *via* the intermediate addition compound of phenylacetylene and phenyldichloroarsine thus:

$$PhC\equiv CH + PhAsCl_2 \longrightarrow \underset{\displaystyle Cl}{\overset{\displaystyle PhC=CH}{|\quad\quad|}}\ AsClPh \xrightarrow{-HCl}$$



(I)

(where R = Ph, R′ = Cl).

By an alternative mechanism the product (I) may be formulated as R = Cl and R′ = Ph.

But oxidation of the arsindole derivative leads to the formation of *o*-carboxydiphenylarsinic acid, the m.p. and mixed m.p. of the oxidation product being identical with that from anthranilic acid and phenylarsenious oxide (Sakellarios, *Ber.*, 1926, **59**, 2552). This proves the validity of formula (I) and also that the arsenic is in cyclic combination.

It was observed that the temperature influenced the nature of the product to a considerable extent; at an elevated temperature the resultant mass comprised of a mixture of solids and liquids of indefinite composition.

1-Phenyl-3-chloroarsindole is a yellow liquid with a greenish tinge, possessing a less offensive odour than 1-chloroarsindole. It gives a yellow crystalline picrate, mercuric chloride addition product and arsonium salts.

EXPERIMENTAL.

1-Phenyl-3-chloroarsindole (I, R = Ph, R′ = Cl).—A mixture of phenylacetylene (10 g.) and phenylarsenious chloride (28 g.) was heated under reflux for 7 hours at 140-50°. The mass gradually darkened with a rise in temperature and at 120-22° there was much evolution of hydrogen chloride. The resultant dark brown viscous mass was treated with absolute alcohol with vigorous stirring, when a yellow crystalline arsenic-free solid, undergoing decomposition on exposure to air, was obtained. The filtrate was heated under reduced pressure till all the alcohol was removed and the resulting brown oil was fractionated and portions boiling (*i*) at 130-55°/12 mm. and (*ii*) at 165-75°/12 mm. were collected. The second fraction, turbid in appearence, was treated with ether, filtered from insoluble matter and the filtrate redistilled at 168-75°/10 mm., when a yellow oil was obtained. [Found : C, 58·1; H, 3.4; Cl, 12·5; As, 25·8; M W. (cryoscopic in benzene), 285·9. $C_{14}H_{10}ClAs$ requires C, 58·2; H. 3·4; Cl, 12·3; As, 25·9 per cent. M.W., 288.5] The *picrate* was crystallised from alcohol, m.p. 115-16°

1-Phenyl-3-chloroarsindole mercurichloride.—Molecular proportions of (I) and mercuric chloride were taken in alcohol and heated till the mixture went completely into solution and then allowed to cool. The mercuric chloride adduct separated out in flakes and was recrystallised from alcohol, m.p. 232-33°. (Found : Hg, 35.6. $C_{14}H_{10}Cl_3AsHg$ requires Hg, 35·7 per cent).

Methiodide of (I) was prepared by heating 1-phenyl-3-chloroarsindole with excess of methyl iodide in a sealed tube at 110° for 14 hours. Excess of methyl iodide was removed in *vacuo* and the resulting brown pasty mass was extracted several times with petroleum ether which on evaporation gave needle-shaped crystals. These were recrystallised from minimum quantity of alcohol, m.p. 152-53°. (Found : As, 17·9. $C_{15}H_{13}ClIAs$ requires As, 17·6 per cent).

Ethiodide derivative was prepared in a similar way with ethyl iodide, m.p. 161° after crystallisation from alcohol in needles. (Found : As, 16·5. $C_{16}H_{15}ClIAs$ requires As, 16·8 per cent).

Oxidation of 1-phenyl-8-chloroarsindole : Formation of o-Carboxydiphenylarsinic Acid.—1-Phenyl-3-chloroarsindole was treated with excess of perhydrol (30 vols.) when a vigorous reaction ensued. After the vigour of the reaction had subsided the whole was evaporated to dryness on a steam-bath. This was repeated several times with fresh additions of perhydrol and the solid obtained was crystallised from water, m.p. and mixed m.p. with Sakellario's compound, 166°. (Found: As, 24·1. Calc. for $C_{13}H_{11}O_4As$: As, 24·5 per cent).

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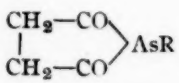
STUDIES IN ORGANO-ARSENIC COMPOUNDS. PART IX. SYNTHESIS OF ARSENIC ANALOGUE OF SUCCINIMIDE.

By Hirendra Nath Das-Gupta.

Arsenic analogue of succinimide has been synthesised by the application of Fittig's reaction to a mixture of succinyl chloride and phenyldichloroarsine in xylene solution. The constitution of the succinylphenylarsine has been established by its reduction to phenyl*cyclo*tetramethylenearsine and the formation of $HgCl_2$-double salt and arsonium derivative.

This paper deals with the synthesis of the arsenic analogue (I, R = H) of succinimide, which could not be prepared from succinic anhyride and arsine. A phenyl derivative (I, R = Ph) of the desired arsine has, however, been prepared by Fittig's reaction from succinyl chloride and phenyldichloroarsine.

Arseno-magnesium compound of the type $AsC_6H_5(MgBr)_2$ is transformed in benzene solution by acetyl chloride into diacetylphenylarsine, $As\,C_6H_5(COCH_3)_2$, a yellow oil, which is oxidised in air and reacts with methyl iodide to form phenyltrimethylarsonium iodide and acetyl iodide. But carbonyl chloride or ethylene dibromide gives unstable compounds, which decompose, yielding arsenobenzene and carbon monoxide in the first case and arsenobenzene and ethylene in the second case (Job and Reich, *Compt. rend.*, 1923, **177**, 56; Job, Reich and Vergnand, *Bull. Soc. chim.*, 1924, **35**, 1404).

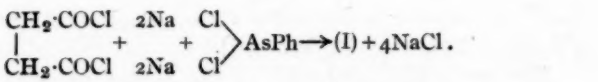


(R = Ph).

(I)

Similarly diphenylarsine reacts vigorously with acetyl chloride in an atmosphere of carbon dioxide to form diphenylacetylarsine (Steinkopf Schubart and Schmidt, *Ber.*, 1928, **61**, 678). The product decomposes on exposure to air and moisture with evolution of heat.

In the present investigation a mixture of succinyl chloride and phenyldichloroarsine was heated with sodium in dry xylene, containing a few c.c. of dry ethyl acetate. The condensation takes place in the following way:



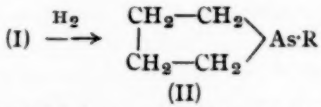
The compound is very unstable as it readily undergoes hydrolysis to succinic acid and phenylarsenious oxide by atmospheric moisture; hence moisture was excluded in its preparation. It has a very corrosive effect on the skin.

The formula (I) was assigned to succinylphenylarsine on the following grounds :

(*i*) It could be hydrolysed to succinic acid and phenylarsenious oxide.

(*ii*) It could be reduced to the known phenyl*cyclo*tetramethylenearsine.

(*iii*) The m.p. and mixed m.p. of the methiodide and mercurichloride of Gruttner and Krause's product (*Ber.*, 1916, **41**, 437) being identical with the same derivatives of the reduction product. The reduction of (I) was effected by sodium and absolute alcohol in toluene solution using the method of Stanley and Adams (*J. Amer. Chem. Soc.*, 1929, **51**, 1515).



(II)

The compound (II) gives a mercuric chloride double salt and arsonium derivatives with alkyl iodides, the 5-membered ring remaining intact, (contrast the action of methyl iodide on diacetylphenylarsine).

EXPERIMENTAL.

Succinylphenylarsine —Dry benzene (50 c.c.), sodium wire (9 g.) and dry ethyl acetate (5 c c.) were taken in a flask, provided with condenser and calcium chloride guard tube and kept for 1 hour. A mixture of succinyl chloride (freshly distilled, 15 g.) and phenyldichloroarsine (22 g.) was then introduced and the reaction started by heat after which the source of heat was withdrawn. The colour of the reaction mixture gradually changed to brown and after the abatement of the initial vigorous reaction the whole was heated for 1 hour on the steam-bath and then kept overnight. The highly viscous product was extracted several times with benzene, filtered till the filtrate was colourless, avoiding the access of moisture. After the removal of the solvent in an atmosphere of dry carbon dioxide, the residual brown oil was fractionated. The portion boiling at 116-20°/10 mm. was redistilled at 119-20°/10 mm. The oil is soluble in the ordinary organic solvents and gives a picrate, m.p. 117°. [Found: C, 50·9; H, 3·9; As, 31·8;

M. W. (cryoscopic in benzene), 230·9. $C_{10}H_9O_2As$ requires C, 50·8; H, 3·8; As, 31·7 per cent. M. W., 236].

Mercurichloride compound (I, $HgCl_2$)was prepared by mixing equimolecular proportion of the two in ether when a white precipitate was obtained. The substance was crystallised from hot alcohol, m.p. 245°. (Found : Hg, 39·4. $C_{10}H_9O_2AsCl_2Hg$ requires Hg, 39·4 per cent).

The Methiodide was prepared by heating in a sealed tube a mixture of succinylphenylarsine and 3 times its volume of methyl iodide for 10 hours at 100°. The resulting brown solution was freed from methyl iodide and the residual pasty oil was dissolved in alcohol and poured on to well cooled ether. The precipitated yellow solid was crystallised from alcohol in needles, m.p. 176°. (Found : As, 19·5. $C_{11}H_{12}O_2IAs$ requires As, 19·8 per cent).

The ethiodide was prepared in a similar manner with ethyl iodide, m.p. 165-67°. (Found : As, 18·9. $C_{12}H_{14}O_2AsI$ requires As, 19·1 per cent).

Reduction of Succinylphenylarsine to Phenylcyclotetramethylenearsine.—A mixture of dry toluene (50 c.c.) and sodium (10 g.) was taken in a three-necked flask, fitted with a separating funnel, a mechanical stirrer and a condenser. The mixture was heated till the sodium melted and then the stirrer was set in motion so that the sodium was granular. The mixture of absolute alcohol (25 c.c.) and succinylphenylarsine was then run in and the whole agitated. A second lot of alcohol (25 c c.) was again added and the mixture stirred till the sodium disappeared. The reaction was completed by heating and the cold reaction mass treated with water and the toluene layer washed successively with water, dilute hydrochloric acid, dilute alkali and finally with water. This was kept overnight with calcium chloride and filtered avoiding as far as possible moisture from the air, since the product decomposes in presence of water when it is distilled. After the removal of the solvent in *vacuo* the residual oil was fractionated, the portion distilling at 120-30°/15 mm. being collected. This was redistilled at 125-30°/15 mm. (Found : As, 36·2. $C_{10}H_{13}As$ requires As, 36·05 per cent).

Methyl cyclo*tetramethylenephenylarsonium iodide* was prepared by the method of Gruttner and Krause (*loc. cit.*), m.p. and mixed m.p. 135-36°. (Found: As, 21·1. Calc. for $C_{11}H_{16}AsI$: As, 21·4 per cent).

Mercurichloride salt, prepared in a similar way, had m.p. and mixed m.p. with Gruttner and Krause's product, 160-62°.

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INDIGOID VAT DYES OF THE ISATIN SERIES. PART III
3-INDOLE-2′-(4′-METHYL)-THIONAPHTHENEINDIGOS.

By Sisir Kumar Guha.

3-Indole-2′-(4′-methyl)-thionaphtheneindigo and its various substituted products, the substituents being present in the indole group, have been prepared and their dyeing shades on cotton and on wool compared with those of the corresponding 5′- and 6′-methyl compounds. The change in colour due to Me-substitution in 4, 5 and 6 positions of the thionaphthene nucleus of 3-indole-2′-thionaphtheneindigo has been found to be produced in the same way as observed by the author in the acenaphthenequinone and the aldehyde series.

The effect of Me-groups in a molecule of thioindigo in its 4 :4′-, 5 :5′- and 6 :6′- position resembles that of the above two series and also isatin series.

In the acenaphthenequinone series, a study of the isomeric methyl-thionaphthene-acenaphthyleneindigos (Guha, *J. Indian Chem. Soc.*, 1932, **9**, 423; 1933, **10**, 679 ; 1936, **13**, 94 ; 1938, **15**, 20) and in the aldehyde series, a study of the isomeric *bis*-(methyl)-thionaphthene-ethyleneindigos and benzylidene-(methyl)-thionaphthenes (Guha, *J. Indian Chem. Soc.*, 1935, **12**, 659; 1937, **14**, 709; 1938, **15**, 359; Auwers and Arndt, *Ber.*, 1909, **42**, 541), has shown that the position of Me-group in the thionaphthene nucleus of the compounds of these two series, is of great influence in deepening or lightening the colour of these isomeric thioindigoid dyes. Of the three positions 4, 5 and 6 of the thionaphthene nucleus, when the Me-group is introduced in 5 position, *i.e.*, *meta* to the indigo chromophore CO, the deepening effect becomes prominent and the colour is lightened when the Me-group enters into 4 and 6 positions, *i.e. ortho* and *para* respectively to CO group.

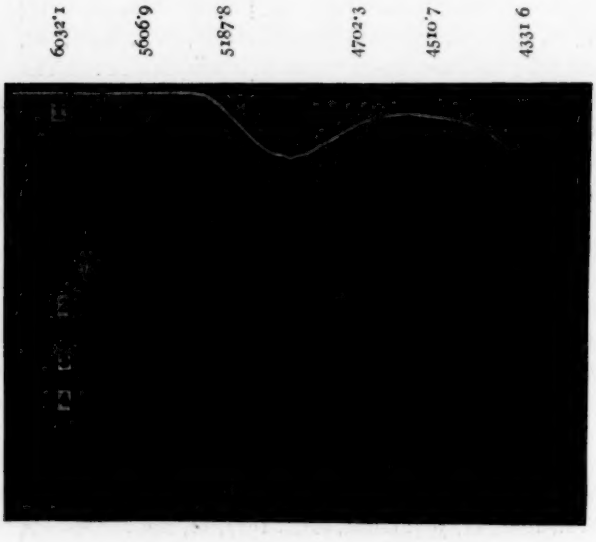
In order to elucidate further the above observation, the present investigation was undertaken. It deals with indigoid dyes obtained by condensing 4-methyl-3-hydroxythionaphthene (*Chem. Zentrl.*, 1928, **99**, **1**, 759; E. P. 279489, U. S. P. 28037; Guha, *J. Indian. Chem. Soc.*, 1938, **15**, 23) with isatin and its various derivatives and the following new dyestuffs have been obtained: 3- indole-, 3- (5-chloro) -indole-, 3- (5-bromo) -indole-, 3 (5 : 7-dibromo) -indole-, 3-(5- bromo-7-nitro) -indole-, 3-(5:7- dinitro) -indole- 2′- (4′-methyl)-thionaphtheneindigo (*cf.* Guha and Basu-Mallick, *J. Indian Chem. Soc.*, 1934, **11**, 395; Guha, *ibid.*, 1937, **14**, 240).

As a result of the study of the properties of these isomeric methyl dyes of the isatin series and the quantitative measurement of some of them in xylene solution (Table I), it has been found that the change in colour due to the Me-substitution in 4,5, and 6 positions of the thionaphthene nucleus

of 3-indole-2′-thionaphtheneindigos (Bezdzik and Friedländer, Monatsh., 1908, **29**, 376. E. P. 17162-06; E. P. 6490-07, G. P. 277358, etc.) is produced in the same way (*vide* figures 1, 2 and 3) as was observed in the other two series (*loc. cit*).

A detailed study of the effect of substitution by Me or Cl on the colour and fastness of thioindigos was recently made by I. G. Farben industrie and the Society of Chemical Industry, Basel. The 4 : 4′-dimethylthioindigo appeared to be particularly fast to light (*Chem. Zentrl.*, 1928, **99, I**, 759 *et seq*; Thorpe and Linstead, " Synthetic dystuff " 1933, p. 203), but nothing has been mentioned there with regard to the colour and the depth of colour of the alkyl thioindigos.

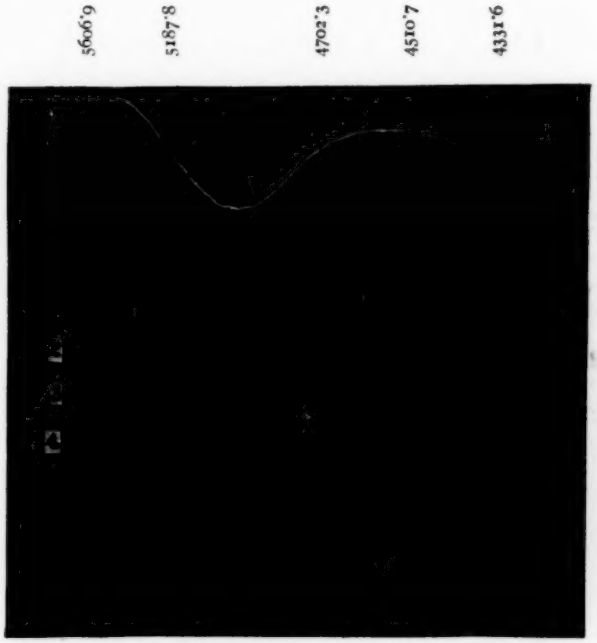
FIG. 1.



Curves 1—4 refer respectively to 3-indole-2-′, -2′-(6′-methyl-, 5′-methyl- and -4′-methyl)-thionaphtheneindigos.

Thioindigo, 4:4′-,5:5′-, and its 6:6′-dimethyl derivatives (Friedländer, *Ber.*, 1906, **39**, 1060 ; E. P. 279489; U. S. P. 28037 ; Auwers and Arndt, *loc. cit.*; Friedlander, 9, 589, D. R. P. 204763, Auwers and Thies, *Ber.*, 1920, **53**, 2285) were prepared and their absorption spectra taken in xylene solution with a view to study the depth of colour and compare the relationship with the dyestuffs of the acenaphthenequinone, aldehyde and isatin series recently studied by the present author. The absorption curves (Fig. 4) of each of these four substances show two maxima. The wave-length was deduced for both the points of each curve and mentioned in Table II. From the curves and wave-lengths the influence of Me-groups in 4:4′- and 6:6′- position of thioindigo have been found to lighten the colour, the maximum effect being in 6:6′-position; the influence in 5:5′- position is to deepen the colour of the

FIG. 2.

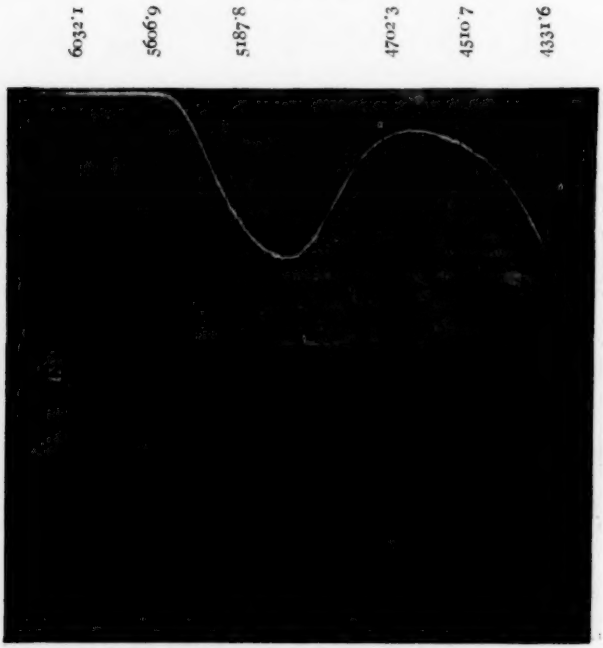


Curves 1—4 refer respectively to 3-(5 : 7-dibromo)-indole-2′-, -2′(4′-, 5′-, and 6′-methyl)-thionaphthenindigos.

parent substance. It may be pointed out here that of the two wavelengths calculated for 6:6′-dimethylthioindigo, the longer one does not indicate the maximum lightening effect (Table II). The effect of Me-groups in a molecule of thioindigo in above positions in general resembles that of the acenaphthenequinone, aldehyde and isatin series (*cf.* Friedlander, *Ber.*, 1916, **49**, 955).

The dyestuffs described in this paper have imparted full shades on cotton from an alkaline hydrosulphite vat, the colour of which is similar to that of the vat obtained from the corresponding 5′-and 6′-methyl derivatives. They dissolve in concentrated sulphuric acid giving a blue solution in each case. These compounds, with the exception of the bromonitro and the dinitro derivatives, dye wool from an acid bath giviing uniformly and fully developed shades. They also develop lustre when rubbed hard. When

FIG. 3.



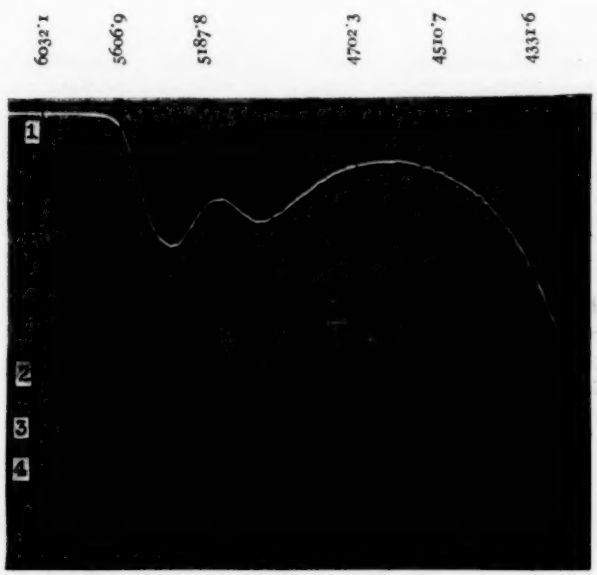
Curves 1. 3—4 refer respectively to 3-(5 : 7-dinitro) indole-2′(4′-methyl-, 5′-methyl-, and 6′-methyl)-thionaphtheneindigos.

Curve 2 refers to 3-(5 :7-dinitro)-indole-2′-thionaphtheneindigo.

heated above 300°, they melt and quickly volatilise. The 4′methyl dyes, prepared from isatin and its 5-chloro- and 5-bromo derivative, have developed colours on cotton and on wool which are deeper than those of the nearly corresponding compounds in the acenaphthenequinone series.

The colours of the dyeings on wool and on cotton obtained from the isomeric methyl compounds of this series are given in Table I for comparison. The 4′-, 5′-, and 6′- methyl derivatives of 3-(5: 7-dinitro)-indole-2′ thionaphtheneindigo were prepared by the present author (Parts I, II and III). The parent compound has now been obtained and the properties investigated. The absorption spectra of this dinitro compound, 3-indole-2′-thionaphtheneindigo (Thioindigo scarlet R) and its dibromo derivative (Ciba Red G) were taken, the curves drawn (Figs. 1, 2 and 3) and also their wave lengths deduced for comparison with their isomeric methyl derivatives (Table I).

FIG. 4.



Curves 1—4 refer respectively to 6 : 6′-, 5 : 5′-, 4 : 4′-dimethylthioindigos and thioindigo.

Table I.

T = Thionaphtheneindigo.

Compounds.	Colour in concentrated H_2SO_4.	Colour on wool.	Colour on cotton.	Absorption maxima.
3-Indole-2′T (Thioindigo Scarlet R)	...	...	...	4892 Å
3-Indole-2′-(4′-Me)-T	Blue	Dark red	Dark red	4885
3-Indole-2′-(5′-Me)-T	Olive brown	Brilliant scarlet red	Light red	4986
3-Indole-2′-(6′-Me)-T	Olive brown	Red	Light red	4880
3-(5-Chloro) indole-2′-(4′-Me)-T	Blue	Dark red	Dark red	...
3-(5-Chloro) indole-2′-(5′-Me)-T	Olive green	Scarlet red	Scarlet red	...
3-(5-Chloro) indole-2′-(6′-Me)-T	Olive green	Red	Red	...
3-(5-Bromo) indole-2′-(4′-Me)-T	Blue	Dark red	Dark red	...
3-(5-Bromo) indole-2′-(5′-Me)-T	Olive green	Scarlet red	Scarlet red	..
3-(5-Bromo) indole-2′-(6′-Me)-T	Olive green	Red	Red	
3-(5 :7-Dibromo) indole-2′-T (Ciba Red G)	...	...	...	4915
3-(5 :7-Dibromo) indole-2′-(4′-Me)-T	Blue	Dull red	Deep red	4910
3-(5 :7-Dibromo) indole-2′-(5′-Me)-T	Olive green	Deep red	Deep red	4998
3-(5 :7-Dibromo) indole-2′-(6′-Me)-T	Olive brown	Light scarlet red	Scarlet red	4897
3-(5-Bromo-7-nitro) indole-2′-(4′-Me)-T	Blue	Dark red (dull)	Dark red	...
3-(5-Bromo-7-nitro) indole-2′-(5′-Me)-T	Deep green	Dark red	Dark red	...
3-(5-Bromo-7-nitro) indole-2′-(6′-Me)-T	Yellowish green	Red	Dark red	...
3-(5 :7-Dinitro) indole-2′-T	Blue	Dark red	Dark red	4950
3-(5 :7-Dinitro) indole-2′-(4′-Me)-T	Blue	Dark red	Dark red	4945
3-(5 :7-Dinitro) indole-2′-(5′-Me)-T	Deep green	Dark red	Dark red	5070
3-(5 :7-Dinitro) indole-2′-(6′-Me)-T	Olive brown	Blood red	Dark red	4927

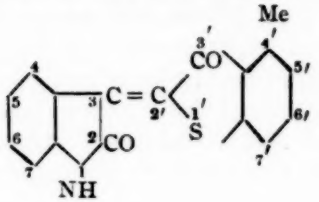
Table II.

Compounds.	Absorption maxima longer.	shorter.
Thioindigo (*cf.* Friedlander and Risse, *Ber.*, 1914, **47**, 1922)	5250 Å	5028 A°
4 :4′-Dimethyl thioindigo	5248	5025
5 :5′-Dimethyl-thioindigo	5615	5296
6 :6′-Dimethyl-thioindigo	5295	4936

EXPERIMENTAL.

4 : 4′-Dimethylthioindigo was prepared by the oxidation in the usual way of a pure specimen of 4-methyl-3-hydroxythionaphthene. It is soluble in pyridine, aniline, xylene, nitrobenzene and chloroform ; moderately soluble in amyl alcohol and acetic acid ; sparingly soluble in alcohol and acetone. The solution in concentrated sulphuric acid is green from which water precipitates the original substance. It crystallises from xylene or nitrobenzene in deep red long rectangles. It melts and volatilises when strongly heated above 305°.

3-Indole-2′-(4′-Methyl)-thionaphtheneindigo.



It separated from isatin (0·588 g.) and 4-methyl-3-hydroxythionaphthene (0·656 g.) in 85 c.c. of glacial acetic acid and 4 c.c. of concentrated hydrochloric acid in silky dark red big needles. The dye (0·898 g.) was crystallised from acetic acid in fine needles. It is moderately soluble in alcohol, difficultly soluble in acetone. It dyes wool in dark red shades from an acid bath and cotton in the same shade from an alkaline hydrosulphite vat. (Found : S, 10·7. $C_{17}H_{11}O_2NS$ requires S, 10·92 per cent).

3-(5-Chloro) indole-2′-(4-methyl)- thionaphtheneindigo was obtained as dark red crystalline precipitate by reacting 5-chloroisatin (0·5445 g.) and 4-methyl-3-hydroxythionaphthene (0·492 g.) in 80 c.c. of glacial acetic acid and 6 c.c. of concentrated hydrochloric acid. The product (0·75 g.) was crystallised from nitrobenzene in fine dark red clusters of needles. It dyes wool in dark red shades from an acid bath and cotton in nearly the same shade from hydrosulphite vat. (Found : Cl, 11·10. $C_{17}H_{10}O_2NClS$ requires Cl, 10·83 per cent).

3-(5-Bromo)-indole-2′-(4′-methyl)-thionaphtheneindigo was prepared from 5-bromo-isatin (0·463 g.) and methylhydroxythionaphthene (0·336 g.) in 85 c.c. of acetic acid and 5 c.c. of concentrated hydrochloric acid. The deep red crystalline substance (0·543 g.) was crystallised in the same way as the preceding compound and it posseses similar properties. (Found : Br, 21·8. $C_{17}H_{10}O_2NBrS$ requires Br, 21·51 per cent).

3-(5:7-*Dibromo*)-*indole*-2′-(4′-*methyl*)-*thionaphtheneindigo* separated in the same shape as the corresponding 5′-and 6′-methyl compounds from 5:7-dibromoisatin (0·61 g.) and 4-methyl-3-hydroxythionaphthene (0·328 g.) in 80 c.c. of acetic acid and 5 c.c. of concentrated hydrochloric acid. The dye (0·792 g.) crystallised from nitrobenzene in bundles of foliated needles. It dyes wool in dull red shades and cotton in deep red shades. (Found: Br, 35·78. $C_{17}H_9O_2NBr_2S$ requires Br, 35·47 per cent).

3-(5-*Bromo*-7-*nitro*)-*indole*-2′-(4′-*methyl*)-*thionaphtheneindigo* was obtained in dark red crystalline precipitate from 5-bromo-7-nitroisatin (0·542 g.) and 4-methyl-3-hydroxythionaphthene (0·328 g.) in 50 c.c. of concentrated hydrochloric acid. The substance (0·738 g.) was crystallised from pyridine in fine small needles. It dyes wool and cotton in dark red shades. (Found : Br, 18·23. $C_{17}H_9O_4N_2BrS$ requires Br, 19·18 per cent).

3-(5:7-*Dinitro*)-*indole*-2′-(4′-*methyl*)-*thionaphtheneindigo* separated as silky deep scarlet red crystalline precipitate from 5:7-dinitroisatin (0·374 g.) and 4-methyl-3-hydroxythionaphthene (0·258 g.) in 40 c.c. of acetic acid and 4 c.c. of concentrated hydrochloric acid. The condensation product (0·57 g.) was crystallised from nitrobenzene in clusters of long rectangles. It is sparingly soluble in xylene and acetic acid. It dyes wool and cotton in dark red shades. (Found : S, 8·01. $C_{17}H_9O_6N_3S$ requires S, 8·35 per cent).

3-(5:7-*Dinitro*)-*indole*-2′-*thionaphtheneindigo* was prepared by heating 5:7-dinitroisatin (0·711 g.) and 3-hydroxythionaphthene (0·45 g.) in 70 c.c. of glacial acetic acid and 4 c.c. of concentrated hydrochloric acid for 20 minutes. The dark red fine small needles (0·25 g.) was collected and crystallised from nitrobenzene. It does not melt below 305°. The dye is soluble in pyridine, aniline, nitrobenzene; sparingly soluble in xylene, benzene, amyl alcohol, acetone, chloroform and alcohol. The solution in concentrated sulphuric acid is blue. It dyes wool in dark red shades from an acid bath and cotton in the same shade from an alkaline hydsosulphite vat. (Found : S, 8·52. $C_{16}H_7O_6N_3S$ requires S, 8·67 per cent).

My thanks are due to Messrs D. K. Bhattacharyya and B. N. Ghose for helping me with spectrograph and microphotometer.

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ESSENTIAL OIL FROM FLOWERS OF *KEWDA* (*PANDANUS ODORATISSIMUS*).

By S. S. Deshapande.

The essential oil from the flowers of *Kewda* contains methyl ether of β-phenylethyl alcohol (about 79%), which imparts the attractive fragrance. The ethyl has been synthesised from the alcohol.

An essential oil extract from the flowers of Kewda in the Hindusthan Aromatics Laboratories has been described (Sadgopal, *J. Soap Perfumery and Cosmetics*, 1937, **10**, 396). An oil (A) isolated from the same source by the present author seems to be different from the former in physical properties and composition.

Oil described by Hindusthan. Aromatics Laboratory.	Oil A.
$n_D^{20°}$ 1·5220	$n_D^{18°}$ 1·4950
$d_{15°}$, 1·0880	$d_{18°}$ 0·9373
and yield per cent. of material used 0·1 to 0·3	0·06

This difference is probably due to the fact that in the Hindusthan Aromatics laboratory the oil was obtained by extraction with solvents and the fats removed by precipitation with alcohol, whereas the present author obtained it by steam distillation.

15 Kilos of flowers yielded 10 c.c. of the crude oil. On distillation under reduced pressure a small portion (about 30%) passed below 70°/12 mm., but the bulk boiled steadily between 70-74°/12 mm., which on redistillation boiled at 68-70°/12 mm. [Found : C, 80·68 ; H, 9·8, OMe (micro), 18·85. $C_{10}H_{14}O$ requires C, 79·95 ; H, 9·39 ; OMe, 20·64 per cent].

Preliminary experiments showed that this constant boiling fraction contains a small amount of unsaturated impurity. The uptake of hydrogen was about 4% of the value required for one double bond. The same value for uasaturation was obtained by permonophthalic acid titration (Böhme, *Ber.*, 1937, **70**, 379).

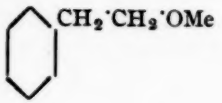
That the group is methoxy and not higher alkoxy was proved by the interaction of the alkyl halide with silver 3 : 5-dinitrobenzoate and the identification of the product as methyl 3 : 5-dinitrobenzoate.

It was not possible to remove the small amount of unsaturated impurity responsible for divergence between theoretical and analytical data. Dilute permanganate was slowly but continuously reduced by the oil.

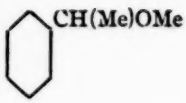
On boiling with excess of hydriodic acid for 5 hours the oil gave an iodide (b.p. 90°/5 mm.), which reacted with pyridine and gave a crystalline additive product, m.p. 164°. (Found : C, 49·89 ; H, 4·23 ; N, 4·75 $C_{13}H_{14}NI$ requires C, 50·1 ; H, 4·5 ; N, 4·5 per cent).

The product is, therefore, $C_{13}H_{14}NI$ and the iodide is C_8H_9I and the oil is mainly the ether C_8H_9OMe or $C_9H_{12}O$ (not $C_{10}H_{14}O$).

Oxidation with permanganate gave benzoic acid. Hence it is either (I) or (II).



(I)



(II)

The iodide (b. p. 90°/5 mm.) reacted with silver 3 : 5-dinitrobenzoate and gave a substance m.p. 106°, identified as 3:5-dinitrobenzoate of β-phenylethyl alcohol.

Hence the substance has the structure (I) and this was confirmed by its synthesis from β-phenylethyl alcohol. The synthetic methyl ether resembles the Kewda component in physical properties and has the characteristic fragrance of Kewda. It reduces permanganate slowly.

	Natural Kewda component.	Synthetic ether having characteristic fragrance of Kewda.
$n_D^{18°}$ ...	1·4950	1·4995
$n_D^{24°}$ ...	1·4930	1·4970
$d_{18°}$ ...	0·9373	d 27° 0·9417
B.p. ...	182-86°/710 mm.	185-86°/710 mm.

The composition of the Kewda oil prepared in the Hindusthan Aromatics laboratory has been given as benzyl benzoate, benzyl salicylate, benzyl acetate, benzyl alcohol, geraniol, santalol, linalol, linalyl acetate, bromostyrol, guaiacol, phenylethyl alcohol, but the methyl ether of β-phenylethyl alcohol which constitutes about 79% of the oil has not been stated to occur.

EXPERIMENTAL

15 Kilos of the outer part of *kewda* flowers freed from the inner part or pollen, were placed on the upper platform of a copper still charged with 39 litres of water. The condensed steam containing the oil was made to pass in succession through layers of chloroform kept in three bottles at descending levels. The chloroform absorbed most of the oil. Distillation was stopped when 20 litres of water were collected. The chloroform containing the absorbed oil was separated from water and the bulk of the oil was obtained on removal of the solvent. A further small amount was obtained by extracting the water with more chloroform, yield 10 c.c. from which 7 c. c. of the component, b. p. 180°-85°/710 mm. or 70-74°/12 mm. was obtained.

Preparation of the Iodide, C_8H_9I.—To 4 g. of the redistilled component were added hydriodic acid (15 c. c., b. p. 127°). The mixture was refluxed with a little red phosphorus at 150° for 5 hours. After removing the unchanged phsphorus, cold sodium hydroxide solution was added until the mixture was slightly alkaline and the heavy oil which separated was extracted with ether. After washing, drying and removing of the solvent the residual liquid was distilled. A small portion boiled at 80-90°/5 mm. but the bulk boiled at 90°/5 mm. The colourless distillate soon acquired red colour due to liberation of iodine.

The iodide reacted with pyridine on warming. The addition product crystallises from alcohol in prisms, m.p. 164°. Analysis, recorded in the introduction, agrees with $C_{13}H_{14}NI$.

Oxidation of the Oil by Permanganate.

To oil (2 g.) was added in instalments potassium permanganate solution (1%, 700 cc.) and the mixture was gently refluxed until the colour of the permanganate was discharged (4 hours). The filtered liquid which was slightly alkaline was concentrated, acidified and extracted with ether, whence benzoic acid (m. p. and mixed m. p. 120°. Found: C, 68·21; H, 5·23 per cent) was isolated.

3:5 Dinitrobenzoate of phenylethyl alcohol was prepared from 3:5-dinitrobenzoyl chloride and phenylethyl alcohol. Crystallising in plates from alcohol it had m.p. 106°. (Found: N, 9·5. $C_{15}H_{22}O_6N_2$ requires N, 8·9 per cent). It showed no depression when mixed with the product of interaction of the iodide (b. p. 90°/5 mm.) obtained from the oil and silver dinitrobenzoate in benzene suspension.

Synthesis of the Ether (I).—Methylation with dimethylsulphate in presence of alkali left much of the alcohol unchanged. Unsatisfactory results were obtained by using sodium methylate and methyl iodide in methyl alcoholic solution. When, however, phenylethyl alcohol (1 mol.) was gently refluxed for 6 hours with methyl iodide (3 mols) and silver oxide (1½ mol.) it was quantitatively methylated. The ether could be distilled without decomposition at ordinary pressure, b. p. 185-86°/710 mm. It is this ether which imparts the attractive fragrance to kewda.

Hammonst (*Compt. rend*, 1901, **138**, 814) obtained this ether by interaction of benzylmagnesium bromide with bromomethyl ether, b. p. 189°/760 mm., but prepared in this way it could not be freed from bromo compounds.

The author expresses his gratitude to Dr. Fürter, Zürich for micro-analysis and to Professor Ruzicka and Dr. Kon for the interest they have shown in the work.

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ON CERTAIN SALTS OF ALKALOIDS.

By U. P. Basu.

A few salts of quinine, emetine and ephedrine have been described.

Owing to the toxicity and various other untoward reactions, the usefulness of salts of alkaloids in therapeutics is restricted. Thus, the injection of emetine hydrochloride is always painful and the drug has a depressant action on the heart muscle and produces cardiac irregularities. Similarly the ephedrine salts exert a considerable depressant action on the cardiac muscle and often (*cf.* Chin and Schmidt, *Medicine*, 1930, **9**, 1) produce toxic symptoms such as nervous excitement and palpitation. Certain esters of quinine are tasteless but there is loss of efficiency due to decreased quinine content. Amongst the non-bitter qninine salts quinine tannate may be mentioned, but it contains only 33 per cent quinine and is slowly decomposed in the intestine. A systematic investigation is being carried out in this laboratory to find suitable salts of alkaloids for therapeutic use (*cf.* Basu, *Science and Culture*, 1937, **2**, 363, 466). But as some of these salts have been prepared and published elsewhere, it has been deemed necessary to publish the results so far obtained.

Experimental.

Emetine-d-camphor-β-sulphonate.—Emetine base in chloroform was prepared by decomposing emetine hydrochloride ($C_{29}H_{40}O_4N_2$, 2 HCl, 7 H_2O) (35 g.) in minimum quantity of double distilled water with dilute caustic soda solution till the mixture was alkaline and then extracting with chloroform. The choloform extract was washed with cold water and to it *d*-camphor-β-sulphonic acid (23·5 g.) was added and well shaken. After a few minutes the solvent was distilled at 40-50° under reduced pressure and the residual mass was cooled and triturated with petroleum ether. A crystalline solid separated out. This was collected and on crystallisation from a mixture of alcohol and dry ether was obtained in clusters of fine needles, m. p. 203-4° (slow heating). (Found: N, 3·01. $C_{29}H_{40}O_4N_2$, $2C_{10}H_{16}O_4S$ requires N, 2·97 per cent).

The salt is very soluble in water and a 9·04% solution, *i.e.* a solution containing the equivalent of 1 grain of emetine base in 1 c.c. (the usual therapeutic dose), has p_H *ca* 4·9. This solution can be sterilised by tyndallisation or by filtration. It possesses the specific action of emetine in amoebic dysentery and is much less toxic and better tolerated than emetine hydro- chloride.

Ephedrine Camphorsulphonate.—A concentrated aqueous solution of ephedrine hydrochloride was made strongly alkaline with sodium hydroxide (50 % solution) and the base was extracted with chloroform. The chloroform solution after being dried with anhydrous sodium sulphate, was treated with molecular proportion of camphorsulphonic acid at room temperature (25°). The white residue, obtained on concentration on the steam-bath at reduced pressure, was crystallised from boiling ethyl acetate in fine silky needles, m. p. 173-74° (with previous shrinkage). (Found: N 3·98. $C_{10}H_{15}ON$, $C_{10}H_{16}O_4S$ requires N, 3·53 per cent). The amount of the ephedrine base present in the salt as estimated by the method of Giffen (*Pharm. Weekblad*, 1936, **73**, 526) (slightly modified) was 41·16 per cent. Theory requires 41·56 per cent.

This ephedrine salt is readily soluble in water, alcohol and chloroform but insoluble in ether, ligroin or petrol ether. A 6·4 per cent solution of the salt had p_H *ca* 5·4. Its physioiogical properties are being determined in detail ; it may be stated that in a case of allergic asthma it gave better relief by a fall in the respiratory rate.

(WITH M. ROY)

Quinine Camphorsulphonate.—The solid that separated when a solution of quinine (1 part) in chloroform was added to a solution of camphorsulphonic acid (2 parts) in the same solvent on slight warming was collected, washed with boiling ethyl acetate and finally crystallised from alcohol diluted with ether, m. p. 218-19°. (Found: N, 3·41 . $C_{20}H_{24}O_2N_2$, $2C_{10}H_{16}O_4S$ requires N, 3·55 per cent). It is readily soluble in water but bitter in taste, and its aqueous solution is acid to litmus (*cf.* Bazzola, *La Sci. del Farm.*, 1937, **8**, 279).

Quinine Mandelate.—An alcoholic solution of mandelic acid (3 g.) and quinine base (3·2 g.) was heated till the solution was neutral. It was cooled and diluted with ether when the salt separated. By dissolution in alcohol and precipitation with ether it was obtained as a flocculent mass, m. p. 189-90°. (Found: N, 6·03. $C_{29}H_{24}O_2N_2$, $C_8H_8O_3$ requires N, 5·88 per cent). It is better and is very sparingly soluble in water.

Quinine-2-oxynaphthoate.—The quiniue base and 2-oxynaphthoic acid were dissolved in chloroform in molecular qroportions and heated under reflux on a steam-bath. After most of the chloroform was distilled off, the salt was precipitated with the addition of petroleum ether, and

repeatedly washed with ether, and finally crystallised from a mixture of chloroform and ether, m.p. 149-50°. It gave low nitrogen value on analysis. The quinine base in the salt as determined by the extraction method was found to be 63·19, whereas theory requires the salt ($C_{20}H_{24}O_2N_2$, $C_{11}H_8O_3$) to contain 63·28%. It is slightly bitter in taste, difficultly soluble in alcohol and insoluble in water.

Quinine-1:1′-methylene-2:2′-dinaphthyl-3:3′-dicarboxylate.—When 1:1′-methylene-2:2′-dinaphthyl-3:3′-dicarboxylic acid(1 part) dissolved in the requisite quantity of sodium carbonate solution was gradually added to a solution of quinine dihydrochloride (molecular proportion), a curdy precipitate was obtained. After standing it was filtered, washed with water and crystallised from absolute alcohol in microscopic canary yellow needles, m.p. 199-200°. (Found: N, 3·61. $C_{20}H_{24}O_2N_2$, $C_{23}H_{16}O_6$ requires N, 3·93 per cent). It is slightly bitter, almost insoluble in water but readily dissolves in chloroform.

In conclusion, the author wishes to express his sincere thanks to Dr. A. Chakravarty, M.R.C.P. for his interest and to Mr. S. Ghosh for his help in the course of this investigation.

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OCCURRENCE OF PSORALEN IN *PHEBALIUM ARGENTEUM*, SMITH.

By P. K. Bose and H. H. Finlayson.

In course of an examination of the essential oil of the Australian plant, *Phebalium argenteum* (N. O. Rutaceae) one of us isolated a colourless crystalline substance, m.p 165° (Finlayson, *Trans. Roy. Soc.*, South Australia, 1928, **52**, 235). This was supposed to be a coumarin derivative on account of its characteristic odour. Further examination could not be undertaken owing to the extremely poor yield of the material. This compound has now been further purified by sublimation at 120-30°/0·05 mm. and subsequent crystallisation from methyl alcohol, when it formed almost transparent prismatic needles, m.p 166-67°. It behaves like a lactone and dissolves in concentrated sulphuric acid with a pale greenish yellow colour. We thought that the compound might be identical with psoralen, which had been isolated from the seeds of *Psoralea corylifolia* (Jois, Manjunath and Rao, *J. Indian Chem. Soc.*, 1933, **10**, 41), and from the leaves of *Ficus Carica* L. (Okahara, *Bull. Chem. Soc. Japan*, 1936, **11**, 389),

Through the kindness of Dr. B. L. Manjunath, to whom our best thanks are due, we obtained a specimen of psoralen which after repeated sublimations in high vacuum and crystallisations melted at 166-67°. The properties of the two compounds were found to be identical and a mixture of the two also melted at 166-67°. The coumarin isolated from *P. argenteum* is thus proved to be psoralen.

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REVIEWS

Das Gallium. By Dr. rer. nat. Erich Einecke, Institut der Christian Albrechts-Universitat—kiel. Published by Verlag Von Leopold Voss, Leipzig 1937. pp. 156, with 17 figures. price R. M. 12.

Though the Chemistry of gallium has not yet been fully investigated even as much as its physics, still the author has succeeded in presenting a critical and comprehensive survey of its properities and behaviour. The study of this rare element has within the last decade received a great impetus from a closer investigation of the mineral germanite of the Tsumete-Mine, which has been found to contain upto 0·75% gallium. The price of this rare metal was still prohibitive costing about 200 Mark per gram till in 1932 the " Vereinigten chemischen Fabrik " at Leopoldshall in Germany started to work out the waste residue obtained in the extraction of copper from the " Kupferschiefer " at Mansfeld. Both gallium and rhenium are now extracted from this residue in appreciably large quantities. This has led to a reduction of the price of gallium to about 10 R. M. only per gram. The element is now accessible to all chemists of moderate means. The appearance of the monograph under review at this time is, therefore, specially welcome and will undoubtedly prove highly useful to all those chemists who are interested in this rare element.

The reader will found in the book a complete reference to all the minerals and rocks in which gallium has been detected, their places of occurrence and their gallium content.

Extraction of the element from zinc blende, germanite, lepidolite and kupferschiefer, and its purification have been described in a clear and concise manner.

A comprehensive account of the various physical properties of the metal such as mechanical, thermal, colloidal, optical including spectral, magnetic, electro-chemical and electrical, has been given with figures and tables. This is followed by a description of its chemical properties.

Various binary and ternary alloys of the metal have been discussed with the help of equilibrium diagrams where necessary.

A more or less complete treatment of almost all the gallium compounds prepared up till now, including those of the organo-metallic type, has been made. Even the volume-chemical, crystallo-chemical and catalytic behaviour of the metal have not been missed.

Analytical methods for the detection and estimation of the element have also received proper attention.

A short discussion of its physiological studies and its uses in arts and industries completes the book.

A special and important feature of the book lies in the fact that it gives indications of still unsolved problems for the benefit of the research-workers on this rare element.

Finally, a complete list of reference to all literatures on the subject,—books, original papers and patents—arranged chronologically and appended at the close, greatly enhances the value of the book.

P. R.

Practical Chemistry for Intermediate Students. By M. L. Sitaraman M.Sc. Published by the Annamalai University. Medeum 8 vo., pp. 1-134.

The book under review is written for the Intermediate students of the Annamalai University and is designed to cover the syllabus of the University. While the book has much to commend itself, it might present some difficulty to the beginner, as the experimental details are not always explained and conclusions are in many cases left to be drawn by the student himself. The last-named feature is undoubtedly conducive to the healthy growth of power of observation and development of critical faculty, but too much brevity might damp the ardour of the beginner and stand in the way of proper appreciation of the object of the experiment. The execution and reproduction of the diagrams could have been improved upon.

On the whole the book will form a useful addition to the list of text-books on elementary Inorganic Chemistry.

S. K. M.

Elementary Inorganic Chemistry. By S. J. Smith. Published by McMillan & Co. Ltd. D. C. 16 vo., pp. 1-391. Price 4½ sh.

The book is meant for the British students preparing for the School Leaving Certificate Examinations. The subject matter is dealt with in an elementary way and the presentation is admirable. Although written for the British students, the book can profitably be adopted as a text-book in Indian Universities for the Matriculation Examination. The theoretical aspects of chemistry have been simply and lucidly treated with explanatory diagrams. The printing and the general get-up of the book leave nothing to be desired.

S. K. M.

THE DETERMINATION OF THE PARACHORS OF INORGANIC SALTS IN SOLUTIONS AND THEIR STRUCTURE. PART II. SOME LITHIUM, SODIUM, RUBIDIUM SALTS AND ATOMIC PARACHORS OF THE ABOVE ELEMENTS INCLUDING CAESIUM.

By Jamiat V. Lakhani and Rustom P. Daroga.

The parachors of some lithium, sodium and rubidium salts have been determined by the solution method and the parachor values of lithium bromide, sodium thiosulphate and sodium bisulphite which have not been previously determined are now found by this method.

For the first group of elements (Li, Na, K, Rb, Cs) the graph obtained by plotting the log of atomic number against the log of parachor (our values) gives a straight line curve, and thus a definite relationship between the atomic number and parachor has been established. From the graph the parachor of cæsium = 150.

In the previous investigation (*J. Indian Chem. Soc.*, 1938, **15**, 37) the authors have shown from the measured parachor of several potassium salts, that by modifying the Hammick and Andrew's equation for calculation of parachors of solutes in solution, the parachors of inorganic salts in solution could be accurately determined. In the present investigation by employing the same modified equation (*loc. cit.*) the authors have determined the parachors of some lithium, sodium and rubidium salts, and the atomic parachors of the above elements including cæsium.

Ray (*J. Indian Chem. Soc.*, 1938, **15**, 43) has determined the parachors of some ammonium salts and of potassium chloride and iodide in aqueous solution, and finds the value of parachor in each case to be less than the $\Sigma[P]$ and is, therefore, of opinion that atomic parachor constants cannot be employed when the elements are in the ionic state. In the calculation of the parachor of salts above referred to, the parachor constant 52·62 for water has been used and the values for ammonium salts are found to be 10 units lower than $\Sigma[P]$. If 54 as the parachor constant of water (Sugden, *J. Chem. Soc.*, 1924, **125**, 1188) is substituted in the calculation, the observed parachors of ammonium salts, however, closely approximate to $\Sigma[P]$.

In recent years anomalies have been found in the parachor constants of elements like palladium, thallium, mercury, iron, cobalt and nickel by Mann and Purdie (*J. Chem. Soc.*, 1935, 1549), Anderson (*J. Chem. Soc.*, 1936, 1283) working on the metallic carbonyl compounds also

TABLE I.

Parachor of sodium salts.

Subst.	x.	D.	γ.	M_M.	P_M.	P_X (Parachors observed by us).	Parchor values obtained by Sugden (*J. Chem. Soc.*, 1929, 1291) from Jaeger's data (*Z. anorg. Chem.*, 1917, **101**, 1).	Atomic parachor of Na	
								deduced from the P (obs.) of the salt by us.	obtained by Sugden (*loc. cit*) from the parachor of the salt.
1	2	3	4	5	6	7	8	9	10
NaCl	0·09723	1·193	84·09	21·939	55·67	142·54	124·8	89·84	72·1
NaBr	0·04190	1·166	78·30	21·561	55·01	156·06	143·8	93·46	77·4
	0·04987	1·196	78·80	22·233	55·38	163·66			
						(mean 159·36).			
$NaNO_3$	0·04072	1·123	74·27	20·731	54·20	58·92			
	0·05152	1·155	74·89	21·449	54·64	66·37	152·9	91·1	61·4
	0·06109	1·173	75·90	22·093	55·56	79·53			
	0·07806	1·212	78·34	23·239	57·06	93·19			
						(182 from graph by extrapolation).			
$Na_2S_2O_3$,	0·05230	1·301	86·80	25·324	59·42	315·0			
$5H_2O$	0·05451	1·312	86·91	25·634	59·65	315·2	Not done	84·9	—
	0·05971	1·339	87·60	26·356	60·24	318·0			
						(mean 316·6)			
$NaHSO_3$	0·04686	1·181	83·33	22·038	56·39	210·0			
at 35°	0·04882	1·190	83·95	22·203	56·49	209·8	Not done	92·6	—
								(mean Na = 90·06)	

observes anomalies in the parachor constants of nickel, cobalt and iron. No explanation has, however, been offered.

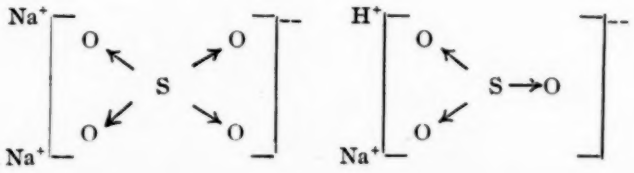
In the case of simple inorganic salts with definite linkages and composition it is reasonable to assume that the parachor constants, determined by the solution method, are free from any structural and packing influences and that the additive character of the parachor is maintained. A new and interesting relationship has been established for the alkaline group of elements between the parachor and atomic number in that the former is connected in a definite manner to the latter which is a fundamental property of the atom.

In Table I are given the data and results obtained with the solution in water of different sodium salts, at the constant temperature of 30°.

It will be noticed that (*a*) the parachor of the two salts—sodium thiosulphate and sodium bisulphite—have not been previously determined on account of the difficulties of fusing them, (*b*) the parachor value of NaCl NaBr, and $NaNO_3$ obtained by the authors are distinctly higher than those obtained by others by the fusion method (*cf.* columns 7 and 8) and (*c*) that the mean atomic parachor of Na = 90·06. In connection with the parachor of sodium chloride, the authors have also calculated this constant from the surface tension and density data of sodium chloride solutions obtained by Harkins and McLaughlin (*J. Amer. Chem. Soc.*, 1925, **47**, 2083), and the mean value calculated was 142·28 in close agreement with the value 142·54 found experimentally by solution method.

In the case of sodium nitrate when the salt is dissolved, there is a change in the volume directly proportional to the concentration, and consequently the parachor has been calculated according to the Hammick and Andrew's equation, and the final value deduced from a series of values of P_x by straight line extrapolation as shown in Fig. 1.

In calculating the *P* of sodium bisulphite the structures given below have been found to fit in with the measured parachor values.



Sodium thiosulphate
P_{obs} = 316·5; P_{Na} = 84·9

Sodium bisulphite
P_{obs} = 20·9 ; P_{Na} = 92·6

The observed parachor of sodium thiosulphate is rather low, as it gives a lower atomic parachor of sodium than the other salts.

In Table II are given the data and the results obtained with two salts of lithium and one of rubidium in water at the constant temperature of 35°.

In this case also, the observed parachor values are higher than those obtained by fusion method. The mean parachor of Li equals 46·8 and of Rb 141·62.

Relation between Atomic Parachor and Atotmic Number of the Metals of the First Group (Li, Na, K, Rb, Cs).

When the parachor constants of these metals as measured here are plotted against their atomic numbers, a smooth parabolic curve is obtained (Fig. 2 *a*). By plotting the logarithms of these two co-ordinates the straight line is obtained (Fig. 2*b*).

The logarithmic curve thus indicates that in the case of alkali metals, parachor is definitely connected with the fundamental property of the atom, namely, the atomic number or the nuclear charge.

The Atomtc Parachor.

Parachor of Lithium.—Sugden (" Parachor and Valency," p. 181) has given the approximate value of parachor for lithium as 50 in error by ± 10 units. The mean value obtained by the authors is 46·8 and fits well on the straight line logarithmic curve discussed above. Consequently Sugden's figure may not be considered in error and may be regarded as correct comparing favourably wlth the value obtained by the solution method.

TABLE II.

Parachor of lithium and rubidium salts.

Substance.	*x*.	*D*.	*Y*.	M_m.	P_M.	P_X. (Parachor observed by us).	Parachor values obtained by Sugden & Wilkins (*J. Chem. Soc.*, 1929, 1291).	Atomic parachor of the metal deduced from the observed parachor of the salts.	obtained by Sugden & Wilkins (*loc. cit.*).
1	2	3	4	5	6	7	8	9	10
LiCl	0·07650	1·096	77·11	19·873	53·7	100·34	97·2 to 99·6	Li = 47·64	45·7
LiBr	0·09095	1·354	83·68	24·26	54·2	112·36	Not done	Li = 45·96	—
RbCl (30°)	0·04282	1·197	78·32	22·401	55·84	194·32	182·8	Rb = 141·62	Rb = (130) ( ) = ± 10

FIG. 1.

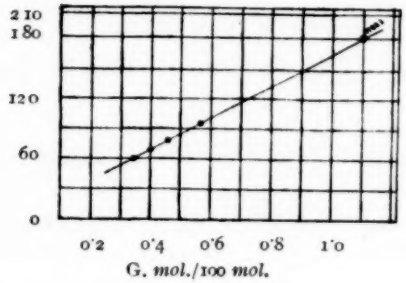


FIG. 2a.

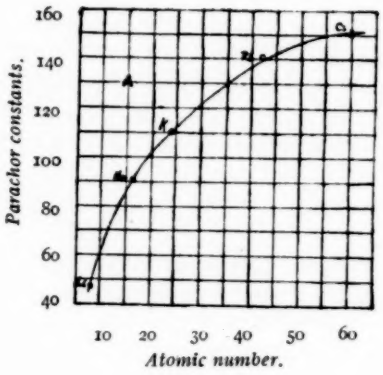
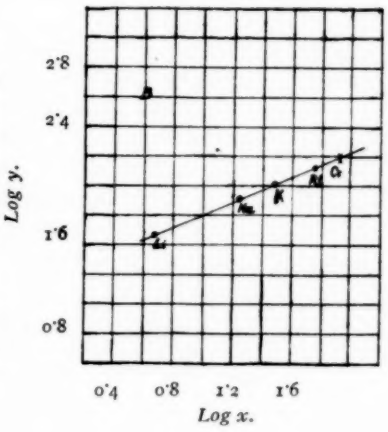


FIG. 2b.



Parachor of Sodium.—In the case of sodium metal Poindexter (*Phys. Rev.*, 1926, **27**, 820) from the surface tension and density data of sodium metal, has arrived at the figure of 97·4 as the parachor constant; Sugden from the study of several salts by fusion method has arrived at the figure of 80 in error by ± 10 units ("Parachor and Valency," p. 181). The solution method gives the mean value of sodium parachor as 90·06. This appears to be more in line with the logarithmic "Parachor—Atomic number" Law, as established above and is in agreement with the higher expected value of Sugden.

Parachor of Rubidium.—The atomic parachor of rubidium is found to be 141·62 and this value fits exactly on the logarithmic curve Fig. 2 *b*.

Parachor of Cæsium.—From the study of fused salts of cæsium, Sugden has deduced 150 as the parachor of the metal atom. If the straight line Fig. 2 *b* is extended it also cuts the parachor co-ordinate at a point corresponding to 150. This, therefore, establishes the value of parachor of cæsium to be 150.

The results obtained by the "solution method" with the salts of alkaline earth metals and their parachor will be discussed in part III of the present series.

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THE CHEMOTHERAPY OF BACTERIAL INFECTIONS. PART I. SYNTHESIS OF SOME DERIVATIVES OF SULPHANILAMIDE.

By K. Ganapati.

In view of the recent discovery of the curative effects of sulphanilamide and many of its derivatives in the infections due to the *cocci* and other bacteria, it was thought worth while to prepare the gold salts of the allylthiourea derivatives of the above mentioned compounds for testing also in the case of tuberculosis. The syntheses of compounds of the general formula p-CH_2 :$CH{\cdot}CH_2{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}C_6H_4{\cdot}R$ are described.

The striking discovery by Domagk (*Z. angew. Chem.*, 1935, **48**, 480 ; *Deuts. Med. Wochs*, 1935, **61**, 250) of the specific action of the red dye, 'Prontosil' (hydrochloride of 4′-sulphamide-2 :4-diaminoazobenzene) against the *β-hemolytic streptococci*, and the subsequent observation that the simple sulphanilamide as also many related sulphur derivatives of the general formula, p-$R{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2{\cdot}R'$, possess a similar chemotherapeutic effect not only against the *streptococci* but also many other bacteria (Buttle, *Proc. Roy. Soc. Med.*, 1937, **31**, 154 ; *Annales Medicochirurgicales*, 1938, **3**, No. 3; Horlein, *Rep. Brit. Assoc. Adv. Sci. Nottingham*, 1937, p. 347), have opened up a new chapter in the chemotherapy of the bacterial diseases. It was, hence, thought worth while to prepare the gold salts of some of their derivatives with a view to study their suitability for treatment of tuberculosis.

One of the requisite properties of the gold salts suitable for the treatment of tuberculosis is that they should be stable to such an extent that after injection they may not easily decompose in the lymph with the rapid liberation of the metal. Though the researches of Kharash (*J. Amer. Chem. Soc.*, 1931, **53**, 3053) have made available many gold compounds wherein the gold atom is directly attached to the benzene ring (just as in the case of arsenic or antimony), yet they are not entirely satisfactory for the present purpose. The aurothio (-SAu) combination which can be derived from a thiophenol or thiourea derivative is a stable union. In view of the fact that the thiophenols in general are more toxic than thioureas and that 'thiosinamine' (ω-allylthiourea) is well known for its bactericidal properties, attempts have now been made to prepare allylthiourea derivatives of the general formula (I) so that their gold salts can be tried in tuberculosis. It will be noticed that such gold salts possess a close relationship with the drug 'lopion' (gold salt of II).

$$p\text{-}CH_2{:}CH{\cdot}CH_2{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH{\cdot}R \quad \ldots \text{ (I)}$$

$$m\text{-}CH_2{:}CH{\cdot}CH_2{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}C_6H_4{\cdot}CO_2Na \quad \ldots \text{ (II)}$$

In this paper some compounds of the general formula (I), synthesised according to the following general scheme, are described :

$$p\text{-}Ac{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2Cl \xrightarrow{R{\cdot}NH_2} Ac{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2NH{\cdot}R \xrightarrow[\text{Hydrolysis}]{} NH_2{\cdot}C_6H_4{\cdot}SO_2NH{\cdot}R \xrightarrow[\text{thiocyanate}]{\text{Allyliso-}} \text{(I)}$$

(III)

In some compounds, the radical R carries an acid grouping so that the final compounds may be used as salts soluble in water. The intermediate compounds of the type (III) are also being pharmacologically investigated. Recently Kolloff (*J. Amer. Chem. Soc.*, 1938, **60**, 950) has also synthesised compounds of this type, a note on the synthesis of some of which has already been published by the author (*Current Science*, 1938, **6**, 608). Such compounds as have been described by Kolloff are, therefore, omitted. All the allylthiourea derivatives so far obtained yield with potassium chloroaurate under the usual conditions the coresponding gold salts.

The synthesis of similar allylthiourea derivatives of diphenyl sulphides, sulphones and sulphoxides will be described in the succeeding communication.

Incidentally, some compounds have been synthesised wherein the carboxyl group (Formula II) is replaced by other acid groupings which are well known for their bactericidal properties.

The compounds are being pharmacologically tested and the results will be described elsewhere.

EXPERIMENTAL.

The yields in all the following experiments are always very good unless otherwise stated.

Attempts to Prepare m-Aminophenyl-ω-allylthiourea, $NH_2{\cdot}C_6H_4{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}CH_2{\cdot}CH{:}CH_2$.—On boiling one molecule of *m*-phenylenediamine with exactly one molecule of allyl*iso*thiocyanate, shining plates, m.p. 95-102°, were obtained, identified as *m*-phenylene-*bis*-(ω-allylthiourea). (Found : N, 18·1. $C_{14}H_{18}N_4S_2$: N, 18·3 per cent). (*cf.* Lellmann, *Annalen*, 1883, **221**, 26). No monoallylthiourea derivative could be isolated.

m-Nitroacetanilide (6 g.) was reduced with iron dust (3 g.) according to the method of Mills (*J. Chem. Soc.*, 1895, **67**, 927) and the ammoniacal solution concentrated on the water-bath. When free from ammonia, to the solution (after filtration) allyl*iso*thiocyanate (3 g.) and sufficient alcohol for solution were added and the whole refluxed for 1 hour on the steam-bath. On allowing to cool, *m*-acetaminophenyl-ω-allylthiourea separated in a crystalline form. Crystallised from dilute alcohol in shining leaflets it had m.p. 182°. (Found: N, 16·6; S, 12·6. $C_{12}H_{15}ON_3S$ requires N, 16·9; S, 12·9 per cent).

The above acetyl compound could be hydrolysed to the corresponding amine by boiling with (1:1) hydrochloric acid for a few minutes; it is fairly difficult to get the hydrochloride quite pure and the free amine is too unstable for purification and analysis.

p-Acetaminophenyl-ω-allylthiourea, $CH_3{\cdot}CONH{\cdot}C_6H_4{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}CH_2{\cdot}CH{:}CH_2$.—As in the case of *m*-phenylenediamine, *p*-phenylenediamine (1 mol) gave on boiling for one hour in alcoholic solution with one molecule of allyl*iso*thiocyanate di-ω-allylthiourea derivative which crystallised from alcohol in prismatic needles, m.p. 200°. (Found: N, 18·4. $C_{14}H_{18}N_4S_2$ requires N, 18·3 per cent).

p-Acetphenylenediamine (5 g.), allyl*iso*thiocyanate (2·6 g.) and ethyl alcohol (40 c.c.) were refluxed on the steam-bath for 1 hour, the solution diluted with water till a turbidity appeared, and allowed to cool when the condensation product separated in chains of rectangular plates. Recrystallised from dilute alcohol it had m.p. 175°. (Found: N, 17·0; S, 12·8. $C_{12}H_{15}ON_3S$ requires N, 16·9; S, 12·9 per cent).

p-Aminophenyl-ω-allylthiourea, $NH_2{\cdot}C_6H_4{\cdot}NH{\cdot}CS{\cdot}NH{\cdot}CH_2{\cdot}CH{:}CH_2$.—The foregoing acetyl compound (2 g.) was boiled for 15 minutes with hydrochloric acid (1:1; 10 c. c.). The solution was allowed to evaporate at room temperature when the aminohydrochloride separated. It crystallised from ethyl alcohol in nodules, m.p. 230°. [Found: N, 14·6; Cl, 25·3; Equiv., 142. $C_{10}H_{13}N_3S$, 2HCl requires N, 15·0; Cl, 25·3 per cent. Eqiv. (diacidic base), 140].

The hydrochloride on decomposition yielded the base which slowly crystallised from dilute alcohol in beautiful rhombic plates, m.p. 118-20°, but on exposure to air it rapidly turns brown and dark. It did not couple with diazotised *p*-sulphanilamide.

p-(ω-*Allylthiourea*)-*cinnamic Acid*, $CH_2:CH\cdot CH_2\cdot NH\cdot CS\cdot NH\cdot C_6H_4\cdot CH:CH\cdot CO_2H$.—A mixture of *p*-aminocinnamic acid (4 g.) and allyl*iso*thiocyanate (2·5 g.) in dilute alcohol (50% ; 100 c.c.) was refluxed on the steam-bath for 1½ hours, cooled, diluted and left overnight. The product which separated crystallised from boiling water in light yellow prisms or plates, m.p. 171°. (Found: N, 10·5 ; S, 12·0 ; Equiv., 264·7. $C_{13}H_{14}O_2N_2S$ requires N, 10·7 ; S, 12·2 per cent. Equiv., 262).

m-(ω-*Allylthiourea*)-*cinnamic Acid*.—*m*-Aminocinnamic acid (2 g.), allyl*iso*thiocyanate (1·5 g.), sodium hydroxide (2*N*, 7 c.c.) and alcohol (30 c.c.) was refluxed for 1½ hours, the solution was cooled, diluted and acidified when the condensation product separated. It was crystallised from dilute alcohol in fine clusters of colourless needles, m.p. 177° (decomp.). (Found: N, 10·8. $C_{13}H_{14}O_2N_2S$ requires N, 10·7 per cent).

p-(ω-*Allylthiourea*) - *phenylsulphamide*, $CH_2:CH\cdot CH_2\cdot NH\cdot CS\cdot NH\cdot C_6H_4\cdot SO_2NH_2$.—Sulphanilic acid or its sodium salt failed to condense with allyl*iso*thiocyanate under the usual conditions. But *p*-sulphanilamide (4 g.), allyl*iso*thiocyanate (2 g.) and alcohol (50 c.c.), refluxed for one hour, yielded the crystalline allylthiourea derivative which was recrystallised from alcohol in shining colourless needles, m.p. 182°. (Found: N, 15·6 ; S, 23·5. $C_{10}H_{13}O_2N_3S$ requires N, 15·5 ; S, 23·6 per cent).

p-(ω-*Allylthiourea*)-*phenylsulphonyl*-p′-*sulphonamidoanilide*.—$CH_2:CH\cdot CH_2\cdot NH\cdot CS\cdot NH\cdot C_6H_4\cdot SO_2NH\ C_6H_4\cdot SO_2NH_2$.—This was prepared as above by boiling equimolecular quantities of 'disulphonamide or deseptal C' (prepared by the method of Gray, Buttle and Stephenson, *Biochem. J.*, 1937, **31**, 727) and allylthio*iso*cyanate in ethyl alcohol. The product crystallised from dilute alcohol, m.p. 180-81° (decomp.). (Found: N, 12·8. $C_{16}H_{18}O_4N_4S_3$ requires N, 13·2 per cent).

p-*Acetaminophenyldimethylsulphonamide*. $Ac\cdot NH\cdot C_6H_4\cdot SO_2NMe_2$.—A mixture of *p*-acetaminobenzene sulphochloride (5 g.) in benzene and a benzene solution (33%) of diethylamine (5 c.c.) were allowed to stand with good shaking for several hours, then filtered and the product, washed well with water, and crystallised from water in beautiful square plates, m.p. 143°. (Found : N, 11·4. $C_{10}H_{14}O_3N_2S$ requires N, 11·6 per cent).

p-*Aminophenyldimethylsulphonamide*, $NH_2\cdot C_6H_4\cdot SO_2\cdot NMe_2$.—This compound is mentioned in patent D.R.P. 607,537 (*Friedlan.* 1937, **21**, 557) and also by Trefouel, Nitti and Bovet (*Annales de l' Inst. Pasteur*, 1937, **58**, 33), but does not appear to have been described anywhere. The foregoing acetyl compound (3·6 g.) was refluxed with hydrochloric acid (5*N*, 12 c.c.) for ½ hour, the clear solution basified with ammonia after cooling and the product which separated was filtered, washed and crystallised

from alcohol in rhombic plates, m p. 172°. (Found : N, 14·0 ; S, 15·7. $C_8H_{12}O_2N_2S$ requires N, 14·0 ; S, 16·0 per cent).

p-(ω-*Allylthiourea*)-*phenyldimethylsulphonamide*, CH_2: $CH{\cdot}CH_2{\cdot}NH{\cdot}CS{\cdot}NH\ C_6H_4{\cdot}SO_2NMe_2$.—Equimolecular quantities of *p*-aminophenyldimethylsulphonamide and allyl*iso*thiocyanate were boiled for 1 hour in alcohol and the resulting product crystallised from dilute alcohol, m.p. 181°. (Found : N, 13·9 ; S, 21·1. $C_{12}H_{17}O_2N_3S_2$ requires N, 14·0 ; S, 21·4 per cent).

p-*Acetaminophenylsulphonyl*- p'- *dimethylamidoanilide*, $CH_3{\cdot}CONH{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH{\cdot}C_6H_4{\cdot}NMe_2$.—*p*-Acetaminobenzenesulphochloride (1 mol.) and *p*-aminodimethylaniline (little over 2 mols., freshly prepared) were mixed together in ether solution with frequent shaking and allowed to stand overnight. The crystalline product which separated was washed with water and recrystallised from alcohol (charcoal) in beautiful plates, m.p. 196°. (Found : N, 12·5. $C_{16}H_{19}O_3N_3S$ requires N, 12·6 per cent).

p-*Aminophenylsulphonyl*-p'-*dimethylaminoanilide*, $NH_2{\cdot}C_6H_6{\cdot}SO_2NH{\cdot}C_6H_4{\cdot}NMe_2$ —This compound has been referred to as ' Uliron ' by Domagk (*Klin. Wochschr.*, 1937, **16**, 1412 ; *cf.* Buttle, *Proc. Roy. Soc. Med.*, 1937, **51**, 156). It is to be noted that Fourneau, Trefonel, Nitti and Bovet (*Compt. rend. Soc. Biol.*, 1938, **127**, 393) assign the constitution *p*-aminophenylsulphonyl-*p'*-amidophenyldimethylsulphonamide to ' Uliron ' (*cf.* Palazzoli and Bovet, *Annales Medico-Chirurgicales*, 1938, **3**, No. 3, p. 93). No description of this compound, however, is available. This was prepared by the hydrolysis (5*N*-HCl) of the above acetyl derivative. It crystallised from alcohol in shining prismatic needles, m.p. 231°. (Found : N, 14·4. $C_{14}H_{17}O_2N_3S$ requires N, 14·4 per cent).

This compound does not condense with allyl*iso*thiocyanate under usual conditions. But on boiling for 5 hours with allyl*iso*thiocyanate it gives the corresponding allylthiourea derivative which crystallised from dilute alcohol in shining rhombic plates, m.p. 161°. (Found : N, 14·1. $C_{18}H_{22}O_2N_4S_2$ requires N, 14·4 per cent).

p-*Aminophenylsulphonyl*-p'-*amidoanilide*, $NH_2{\cdot}C_6H_4{\cdot}SO_2NH{\cdot}C_6H_4NH_2$. —A mixture of *p*-acetaminophenyl sulphochloride (4 g.), *p*-acetphenylenediamine (5·5 g.) and water (100 c.c.) was well ground in a mortar for 3 hours, filtered and the product washed. *p*-Acetaminophenylsulphonyl-*p'*-amidoanilide thus obtained, is very sparingly soluble in all solvents and was purified by dissolving in dilute sodium hydroxide and precipitating with acetic acid (m.p. about 320°). This product (2·5 g.) was boiled with hydrochloric acid (6 *N*, 25 c.c.) for 1 hour, and the clear solution made just alkaline with ammonia when on cooling the amine separated in fine

needles or nodules. Crystallised from dilute alcohol in clusters of woolly needles it had m.p. 155°. (Found : N, 16·1. $C_{12}H_{13}O_2N_3S$ requires N, 16·0 per cent).

On boiling the above amino compound with 2 molecules of allyl*iso*-thiocyanate a product, which separated as oil and solidified on standing and scratching, crystallised from dilute alcohol, m. p. 175°. [Found : N, 15·21 ; S, 18·1. $C_{16}H_{18}O_2N_4S$ (monothiourea derivative) requires N, 15·5 ; S, 17·7 per cent. $C_{20}H_{23}O_2N_5S_3$ (dithiourea derivative) requires N, 15·2 ; S, 20·8 per cent].

p-*Acetaminobenzenesulphonyl*-m′-*amidocinnamic Acid*, $Ac{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2NH{\cdot}C_6H_4{\cdot}CH : CH{\cdot}CO_2H$.—*m*-Aminocinnamic acid (3·2 g.), *p*-acet aminobenzene sulphochloride (2·3 g.) and water (100 c. c.) were ground together and allowed to stand for 4 hours with frequent shaking. The insoluble residue was filtered and crystallised from dilute alcohol in fine shining needles, m.p. 231° (decomp.). (Found : N, 7·7. $C_{17}H_{16}O_5N_2S$ requires N, 7·8 per cent). The condensation can also be effected in presence of alkali by using molecular proportions of the reactants.

p-*Aminobenzenesulphonyl*-m′-*amidocinnamic Acid*.—The hydrolysis of the above acetyl compound with hydrochloric acid was not satisfactory. The alkaline hydrolysis by the following method gave far better results. The acetyl compound was dissolved in about 40% sodium hydroxide and warmed on the water-bath for 10 minutes. It was then diluted with a little water and warmed further for about 20 minutes. On cooling and carefully acidifying the alkaline solution, the amino acid was precipitated. It crystallised from water in needles or from dilute alcohol in silky needles or rhombic plates, m.p. 213°. (Found : N, 8·4, 8·7 ; S, 9·8. $C_{15}H_{14}O_4N_2S$ requires N, 8·8 ; S, 10·1 per cent).

p-*Acetaminobenzenesulphonyl*-p′-*amidocinnamic Acid*.—This was prepared exactly as the above described *m*-isomer. It crystallised from dilute alcohol in light yellow nodules, m.p. 252° (decomp.). (Found : N, 7·4. $C_{17}H_{16}O_5N_2S$ requires N, 7·8 per cent).

p-*Aminobenzenesulphonyl*-p′-*amidocinnamic acid* was prepared as the *meta* isomer by the alkaline hydrolysis of the corresponding acetyl compound. Crystallised from dilute alcohol in prismatic needles it had m. p. 239° (decomp.). (Found : S, 9·9. $C_{15}H_{14}O_4N_2S$ requires S, 10·1 per cent).

m-Aminomandelic acid undergoes a similar condensation and the product will be described in the succeeding communication.

p-*Acetaminobenzenesulphonamidoethyl Acetate*, $Ac{\cdot}NH{\cdot}C_6H_4{\cdot}SO_2NH{\cdot}CH_2{\cdot}CO_2C_2H_5$.—To a solution of glycine ester hydrochloride (5·5 g.) and

sodium hydroxide (2*N*, 25 c.c.) was added *p*-acetamidobenzene sulphochloride (5 g.) dissolved in alcohol (50 c. c.). After allowing to stand overnight, the solution was concentrated and the condensation product isolated by cooling. It crystallised from dilute alcohol in fine needles, m. p. 129°. (Found: N, 9·3; S, 10·3. $C_{12}H_{16}O_5N_2S$ requires N, 9·3; S, 10·7 per cent).

p-*Aminobenzenesulphonamidoacetic Acid Hydrochloride*, $NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot CH_2 \cdot CO_2H$, HCl.—The above ester (3·5 g.) was refluxed for 1 hour with hydrochloric acid (5 *N*, 12 c. c.). The solution was concentrated when the crystalline hydrochloride separated. It was crystallised from alcohol in nodules of fine needles, m. p. 172° (decomp.). (Found: N, 9·9; Cl, 12·8. $C_8H_{11}O_4N_2ClS$ requires N, 10·5; Cl, 13·3 per cent). The free base, m. p. 151°, has been described by Kolloff (*loc. cit.*).

Condensations with other amino-acids such as glutamic acid, leucine, aspartic acid, etc., will be published later on.

p-*Aminobenzenesulphonamidobenzene-p'-sulphonic Acid*, $NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot SO_3H$.—The preparation of this compound was described by the author (*loc. cit.*) in a note and recently Kolloff (*loc. cit.*) has described its synthesis; the details are omitted here.

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CHEMICAL EXAMINATION OF THE SEEDS OF *CLEOME VISCOSA*, LINN. PART I THE CONSTITUENTS.

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A new unsaturated acid, $C_{27}H_{52}O_3$ named viscosic acid and a new flavone, $C_{15}H_6O_2(OH)_3(OMe)$, named viscosin have been isolated from the seeds of *Cleome viscosa*. Tannins, reducing sugars, and myristic and palmitic acids have also been detected.

Cleome viscosa, Linn. (N.O. Capparideæ) which is called Hurhur or Hulhul or Kanphute in Hindusthani, Hurhuriya in Bengali, is a common plant found throughout tropical India, Ceylon and other warmer climates and has been long in use in India as a domestic remedy. According to Moodeen Sheriff, the seeds are anthelmintic, rubeficient and vesicant and in this respect they are regarded much superior to the mustard seeds and equal to the European mustard. According to O'Shaughnessy the whole plant is used in counter-irritation and blistering in Cochin China (Kirtikar and Basu, "Indian Medicinal Plants," 1918, **I**, 98). In the United States, the roots are said to be used as a vermifuge. According to Lindley the seeds are given occasionally in fevers and diarrhoea.

Cleome pentaphylla has been the subject of a previous investigation (Misra and Dutt, *Proc. Nat. Inst. Sci. India*, 1937, **3**, 45), but nothing is known regarding the constituents of *C. Viscosa*.*

From the benzene extract of the seeds of *C. Viscosa* we have been able to isolate a fixed oil (36·59%) which on standing deposited some crystalline matter, and this has been found to be a mixture of myristic acid, palmitic acid and a new acid, *viscosic acid*. From the alcoholic extract of the seeds a new flavone, called by the authors "*viscosin*" (yield 0·04%), tannins, reducing sugars, as also viscosic acid have been isolated. Viscosic acid, m.p. 97°, seems to be an unsaturated fatty acid having the molecular formula, $C_{27}H_{52}O_3$. Although it does not decompose bicarbonate, it can be titrated with caustic potash solution in alcohol and turns blue litmus red in alcoholic medium. It decolourises bromine in chloroform, but attempts to prepare a crystalline bromo derivative have failed. Oxidation with cold dilute potassium permanganate solution yielded a dihydroxy acid, $C_{27}H_{54}O_5$, and obviously it contains one double bond. It has got an iodine value of 22·5 (Wijis' method).

* These two species of cleome are known in Hindusthani by the same name "Hurhur", and they are much confused one with the other in local markets.

Viscosin, m.p. 294-95° (decomp.), has the molecular formula $C_{16}H_{12}O_6$ and contains no water of crystallisation. A preliminary examination readily reveals its nature as a colouring matter belonging to the flavone group. It gives a dark green colour with alcoholic ferric chloride and forms a triacetyl derivative. It contains one methoxy group. These facts indicate that viscosin is a monomethoxytrihydroxyflavone, which is stable to atmospheric oxidation in alkaline solution. An alcoholic solution of viscosin (1%) was found to have a well-defined absorption band between wave-length 4100 and 4620 Å, having the absorption maximaat 4425Å.

A thorough and detailed study of the physiological action of viscosin with special reference to anthelmintic properties will be undertaken at the School of Tropical Medicine, Calcutta. Further work on the subject is in progress.

EXPERIMENTAL.

An authentic sample of the seeds of *Cleome viscosa* was obtained from the Punjab, dried in shade and was crushed to a fine powder. When burnt completely it left 7·10% of a dirty white ash consisting of 13·33% of water-soluble and 86·67% of water-insoluble inorganic material. Besides silica the following ions were detected in the ash: K, Fe, Ca, Mg, Cl, SO_4, PO_4, CO_3.

In order to form an idea of the percentage and nature of the soluble portions of the drug, a sample of the dried and crushed seeds (25 g.) was successively extracted with various solvents in a Soxhlet's apparatus, when the following amounts of extract dried at 100° were obtained.

Benzene Extract (36·59%).—A reddish yellow oil was obtained from which some waxy material separated.

Alcoholic Extract (2·15%).—A yellowish brown mass was obtained, which is soluble in alkalis with light yellow colour. It gave a dark green colouration with alcoholic ferric chloride and reduced Fehling's solution and gave a flocculent precipitate with alcoholic lead acetate.

Chloroform Extract (0·6%) and *Acetone extract* (0·24%) were brown aud gave ferric chloride reaction and a yellow flocculent precipitate with alcoholic lead acetate as well as with basic lead acetate.

The powdered material (4·2 kg.) was repeatedly extracted with benzene, till a portion of it was found to leave no oily residue on complete removal of the solvent. The reddish yellow oil (800 g.) on standing deposited

some dirty white crystalline matter (A) which was filtered, washed well with benzene, dried and then taken up with ether. The fraction, insoluble in ether, was crystallised twice from ethyl alcohol and then repeatedly from methyl alcohol when white needles of viscosic acid melting sharply at 97° were obtained (yield, 0·1%). Viscosic acid is soluble in ethyl and methyl alcohols, acetone, ethyl acetate, easily so in chloroform and benzene and insoluble in ether and water. It does not reduce Fehling's solution. It dissolves in cold concentrated sulphuric acid, gives no colour with ferric chloride and does not form oxime or acetyl derivatives. [Found : C, 76·31, 76·26 ; H, 12·32, 12·50. M.W. (from lead salt), 437 ; Equiv., 442. $C_{27}H_{52}O_3$ requires C, 76·41 ; H, 12·26 per cent. M.W. 424 ; Equiv., 424).

Sodium Salt.—The neutralised solution of the acid when concentrated to about 30 c.c. and when left overnight deposited the sodium salt as white needles which, when recrystallised from alcohol, melted at 129-30°. It is insoluble in water.

Lead Salt.—The acid (1 g.) was dissolved in hot alcohol and an alcoholic solution of lead acetate was added drop by drop when a flocculent white precipitate was obtained which was collected and recrystallised from *iso*amyl alcohol in white crystalline powder, m.p. 138°. It is easily soluble in pyridine, amyl and *iso*amyl alcohols and less so in ethyl and methyl alcohols, insoluble in acetone, benzene and ethyl acetate. [Found : Pb, 20·04, 19·85. $(C_{27}H_{51}O_3)_2$ Pb requires Pb, 19·66 per cent].

Oxidation of Viscosic Acid.—Viscosic acid (1 g.) was suspended in 100 c.c. of caustic potash solution (5%) and a dilute solution (2%) of potassium permanganate was added slowly followed by vigorous shaking till it was in slight excess and left overnight. The precipitated manganse dioxide was dissolved by passing a current of sulphur dioxide, the insoluble white precipitate filtered and dried and extracted by hot alcohol repeatedly. The insoluble precipitate was of unchanged viscosic acid. The soluble portion was crystallised from methyl alcohol and finally from ethyl acetate. The yield of the hydroxy acid was poor but when the oxidation was carried out in pyridine solution the yield improved. It melted at 127°. (Found : C, 70·69 ; H, 11·58. $C_{27}H_{54}O_3$ requires C, 70·74 ; H, 11·79 per cent).

Isolation of Myristic and Palmitic Acids.—The ether-soluble portion of the crystalline matter (A) on concentration deposited a brown substance which was purified by boiling with animal charcoal in methyl alcohol and after recrystallisation from the same medium, it was obtained in the form of white waxy crystals (6·2 g.), m.p. 48° (yield 0·15%). It was acidic towards litmus. Fractionally crystallised from a mixture of alcohol and water for a

number of times, it was separated into two fractions having constant melting points of 54° and 62° respectively, which remained undepressed when mixed melting points were taken with authentic specimens of myristic and palmitic acids.

The first fraction gave the following analytical data, which are in agreement with those of myristic acid. [Found : C, 73·42, 73·61 ; H, 12·04, 12·13. M. W., 240 (by titration). Calc. for $C_{14}H_{28}O_2$: C, 73·68 ; H, 12·28 per cent. M. W., 228.]

The second fraction gave analytical data corresponding to those of palmitic acid. (Found : C, 75·12, 74·93 ; H, 12·16, 12·63. M W., 268. Calc. for $C_{16}H_{32}O_2$: C, 75·00 ; H, 12·50 per cent. M. W. 256).

The mixture, m.p. 48°, contains equal proportions of myristic and palmitic acids (*cf.*, Allen " Commercial Organic Analysis " 1914, **II**, 385).

Isolation of Viscosin.—The defatted seeds were freed completely from benzene and then extracted exhaustively with boiling ethyl alcohol. The combined alcoholic extracts, on concentration and prolonged standing deposited minute crystals, which were filtered off and washed with benzene. Purification of this substance by recrystallisation gave viscosic acid, m.p. 97°.

The mother-liquor was further concentrated, freed from moisture and extracted with petroleum ether in order to remove oil. The insoluble portion was dissolved in alcohol and the alcoholic solution was treated with an excess of lead acetate. The lead salt was collected and decomposed in alcoholic suspension by sulphuretted hydrogen. Lead sulphide was filtered off and the filtrate was concentrated. On dilution with water tannins and a yellow sustance were precipitated. The colouring matter was freed from tannins by extracting with boiling water and recrystallising from the same medium for a number of times and finally from aqueous methyl alcohol which deposited pale yellow hexagonal plates. Viscosin was finally crystallised from aqueous *iso*propyl alcohol. It darkens at 285° and melts with decomposition at 294-95°, yield 1·6 g.

The filtrate from the lead salt of viscosin was freed from lead and concentrated. The residue gave tests for reducing sugars and gave an osazone, m.p. 202°.

Viscosin is easily soluble in ethyl, methyl and *iso*propyl alcohols, acetone, ethyl acetate, glacial acetic acid, pyridine; less so in chloroform, benzene, nitrobenzene and in hot water ; insoluble in petroleum ether, toluene, ether and in cold water. It dissolves in sodium carbonate solution and in caustic alkalis with a bright yellow colour and is precipitated on

acidification. It dissolves in cold concentrated sulphuric acid with a green colour (which turns to orange-yellow on warming), in concentrated nitric acid with red colour and in concentrated hydrochloric acid with a pale yellow colour. With alcoholic ferric chloride it gives a dark green colour and with alcoholic lead acetate a deep yellow precipitate, and a chocolate salt with silver nitrate. The yellow solution of viscosin in warm glacial acetic acid does not change colour on the addition of concentrated sulphuric acid, but becomes greenish-black on the addition of a drop of ferric chloride. It does not reduce Fehling's solution and is non-glucosidic in nature. Treatment of a methyl alcoholic solution with megnesium and hydrochloric acid gives a bright red solution showing that it is a colouring matter of the flavone group. [Found : C, 63·93, 63·87 ; H, 3·90, 4·2 ; OMe, 10·71, 10·56. M. W. (ebullioscopic in methyl alcohol), 312. $C_{16}H_{12}O_6$ requires C, 64·00 ; H, 4·0, OMe, 10·30 per cent. M. W. 300). No loss of weight occurred when the air-dried sample was heated at 120°.

The *lead salt* is a bright yellow amorphous mass. (Found : Pb, 26·1 ; $C_{32}H_{22}O_{12}Pb$ requires Pb, 25·72 per cent).

The *silver salt*, prepared in the usual manner, was obtained as a chocolate powder. (Found : Ag, 27·24. $C_{16}H_{11}O_6Ag$ requires Ag, 26·49 per cent).

Triacetylviscosin was obtained by acetylating viscosin (1 g.) with acetic anhydride (10 c.c.) and fused sodium acetate (2 g.) in the usual manner. It forms soft white shining needles, m.p. 222-23°. It is easily soluble in ethyl and methyl alcohol, glacial acetic acid, acetone and ethyl acetate, and insoluble in benzene, petroleum ether, chloroform and water. It gave no colour with alcoholic ferric chloride. (Found : C, 62·09 ; H, 4·46. $C_{22}H_{18}O_9$ requires C, 61·97 ; H, 4·22 per cent).

One of the authors (M. P. G.) is highly grateful to the Kanta Prasad Research Trust of the Allahabad University for the grant of a scholarship which enabled him to carry out the investigation.

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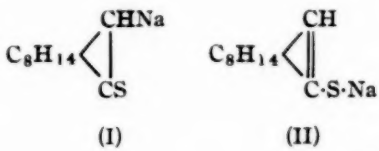
STUDIES IN THE CAMPHOR SERIES. PART V. SOME DERIVATIVES OF *ISO*NITROSOTHIOCAMPHOR.

By Dines Chandra Sen.

The formation of *iso*nitrosothiocamphor on the basis of its reacting in the thiolic phase takes place by a rearrangement involving the migration of the nitroso group from S to C. *iso*Nitrosothiocamphor can give rise to three optical isomers (*d*, *l* and *dl*), each of which in turn should give rise to two geometrical isomers.

In a previous communication (Sen, *J. Indian Chem. Soc.*, 1935, **12**, 752) it has been suggested that the formation of *iso*nitrosothiocamphor (IV, VIII, IX) from *iso*amyl nitrite and sodio thiocamphor takes place through *C*-sodio derivative of thiocamphor (I). But subsequent observations have shown that sodiothiocamphor always reacts in the thiolic phase (II) (Sen, *Science & Culture*, 1938, **4**, 135).

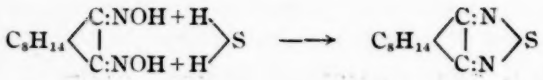
The formation of *iso*nitrosothiocamphor according to this assumption takes place by a rearrangement involving the migration of the nitroso group from sulphur to carbon :



(I) (II)

(III) (IV)

The action of hydroxylamine hydrochloride on *dl-iso*nitrosothiocamphor in presence of either sodium hydroxide, or sodium acetate or pyridine gives rise to three compounds, (1) α-camphorquinone dioxime, m.p. 201°, (2) β-camphorquinone dioxime, m.p. 248° and (3) bornylene 1:2:5-thiodiazole, m.p. 218°. The formation of the thiodiazole (V) evidently takes place through the agency of sulphuretted hydrogen which is formed at the first stage of the reaction. The structure has been confirmed by its direct formation from β-camphorquinone dioxime and sulphuretted hydrogen.

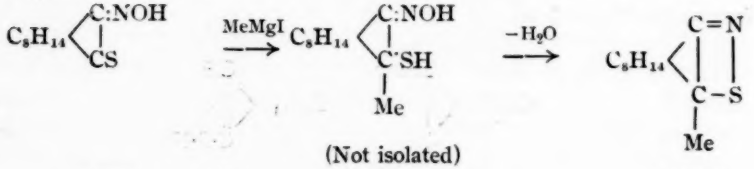


(V)

*iso*Nitrosothiocamphor can give rise to three optical isomers (*d*-, *l*-, and *dl*-) each of which may give rise to two geometrical isomers (*cis*- and *trans*-).

*dl-iso*Nitrosothiocamphor has been prepared from *dl*-thiocamphor and the *l*-isomer from *l*-thiocamphor. But the *d*-isomer could not be prepared owing to the inaccessibility of *d*-thiocamphor. Both these isomers are apparently *cis*-compounds because both of them furuish α- and β-camphorquinone dioximes and bornylene-1:2;5-thiodiazoles on further condensation with hydroxylamine.

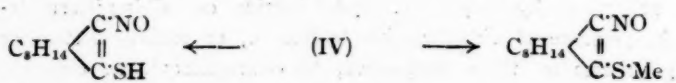
Magnesium methyl iodide reacts with *dl-iso*nitrosothiocamphor giving rise to a compound (VII) which is regarded as the anhydride of *iso*nitroso-methylthioborneol (VI). This reaction also points to the *cis*-structure of *iso*nitrosothiocamphor. It does not give a yellow lead salt nor does it decolourise iodine, showing the absence of any thiol group.



(Not isolated)

(VIII) (VI) (VII)

By the action of methyl iodide and sodiun ethylate on *dl-iso*nitroso-thiocamphor, *S*-methylnitrosothiocamphor (X) is obtained as a blue liquid, b.p. 95-96°/6 mm., which can be hydrolysed by boiling dilute hydrochloric acid to methylmercaptan and *iso*nitrosocamphor. The above reactions indicate that *iso*nitrosothiocamphor can behave both in the thiolic (IX) and thioketonic (VIII) phasse:



(IX) (X)

On hydrolysis with fuming hydrochloric acid in presence of formaldehyde, *iso*nitrosothiocamphor gives mainly camphorquinone, m.p. 198°, and a small quantity of monothiocamphorquinone, m.p. 196°. These

latter gave β-camphorquinone dioxime, m.p. 248° as one of the products on treatment with hydroxylamine (*cf.* Lapworth, *J. Chem. Soc.*, 1907, **91**, 1134).

FIG. 1.

*dl-iso*Nitroso in EtOH (1 g. in 100,000 c.c.).

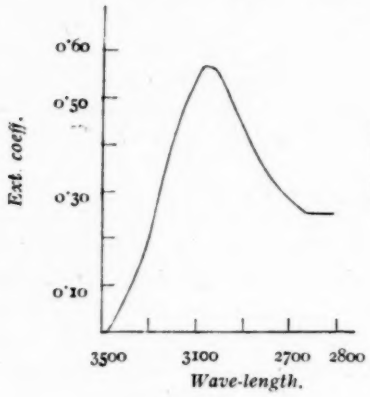


FIG. 2.

*l-iso*Nitroso in EtOH (1 g. in 100,000 c.c.).

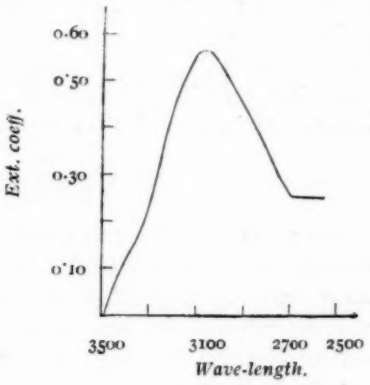


FIG. 3.

*l-iso*Nitroso in EtOH (1 g. in 50 c.c.).

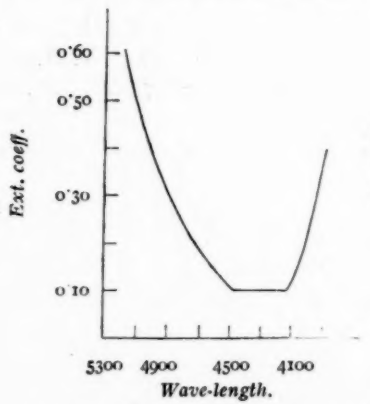
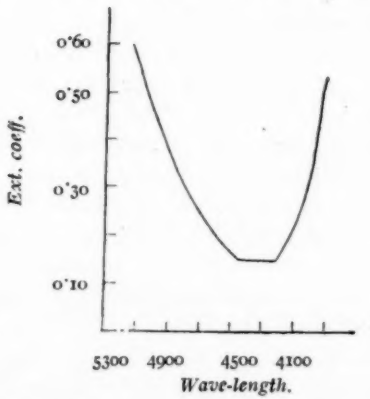


FIG. 4.

*dl-iso*Nitroso in EtOH (1 g. in 50 c.c.).



*l-iso*Nitrosothiocamphor shows dimorphism and dichroism, unlike its *dl*-isomer. In the solid state *l-iso*nitrosothiocamphor is pink, but dissolves in organic solvents with a blue colour. The blue alcoholic solution, if rapidly cooled, gives prismatic crystals, whereas on slow cooling the octahedral form is obtained. The *dl*-isomer is blue both in the solid state and in solution.

The colour of the two isomers during crystallisation from different

solvents under different conditions remained unchanged, and their preparation under identical experimental conditions excludes the possibility of any structural difference. The difference in certain physical properties between isomers of optically active substances is too wellknown. Such difference in colour in optically active isomeride is not very common and deserves mention in this connection. The structural identity of the two isomers in solution has been established by their absorption spectra. Absorption spectra of the two substances at different dilutions show that though there are slight differences in the values of the extinction coefficients, the nature of the curves is the same in both cases (*vide* Figs. 1-4).

EXPERIMENTAL.

Action of Hydroxylamine Hydrochloride on iso*Nitrosothiocamphor.*—*l-iso*Nitrosothiocamphor (10 g.) was dissolved in pyridine (50 c.c.) and heated with hydroxylamine hydrochloride (5 g.) on the water-bath. In a few minutes the blue solution became colourless. The product on being poured into water (500 c.c.) gave a white precipitate, which crystallised from hot alcohol and was identified by mixed m. p. as β-camphorquinone dioxime. (Found : N, 14·22. Calc. for $C_{10}H_{16}O_2N_2$: N, 14·29 per cent). The filtrate from the above on concentration gave α-bornylene 1:2:5-thiodiazole as white needles, m.p. 221°, readily soluble in ether and benzene. (Found : C, 61·93 ; H, 7·43 ; S, 16·39. $C_{10}H_{14}N_2S$ requires C, 61·85 ; H, 7·22 ; S, 16·49 per cent). $[\alpha]_D^{34} = +75°·27$, in 0·31% alcoholic solution. The mother liquor on further concentration gave α-camphorquinone dioxime, m.p. 201°.(mixed m.p remaining undepressed). (Found: N, 14·28. Calc. for $C_{10}H_{16}O_2N_2$: N, 14·29 per cent). Identical products were also obtained in alcoholic solution with hydroxylamine liberated from its hydrochloride by sodium hydroxide or acetate.

*dl-iso*Nitrosothiocamphor on similar treatment gave a mixture of α- and β-camphorquinone dioximes and bornylene-*dl*-1:2:5-thiodiazole, m.p. 218°. (Found : S, 16·42. $C_{10}H_{14}N_2S$ requires S, 16·49 per cent).

The same thiodiazole was also prepared by the action of sulphuretted hydrogen on β-camphorquinone dioxime. β-Camphorquinone dioxime (10 g.) was dissolved in alcohol (100 c. c.) and treated with a saturated solution of sodium acetate (10 g.). Sulphuretted hydrogen was passed through the mixture for 5 hours. The solution was poured into water (500 c. c.) and the separated solid fractionally crystallised from alcohol in white needles, m. p. 218°. The compound was found to be remarkably stable towards potassium permanganate both in acid and alkaline solutions in the cold, but was reduced by zinc and acetic acid to bornylene diamine.

Reduction of Bornylene-1:2:5-thiodiazole.—The above thiodiazole (5 g.), glacial acetic acid (25 c. c.) and zinc dust (5 g.) were heated on a water-bath for 2 hours and filtered. The filtrate after dilution with water (100 c. c) was made alkaline with dilute sodium hydroxide. The solution was extracted with ether. The extract was dried over anhydrous sodium sulphate and the ether evaporated. The residual brown viscous liquid on treatment with a few c. c. of fuming hydrochloric acid gave bornylene diamine hydrochloride which crystallised from alcohol as white shiny needles, m. p. 287-88°. (Found : C, 50·35 ; H, 8·5 ; Cl, 30·02. $C_{10}H_{20}N_2Cl_2$ requires C, 50·20 ; H, 8·37 ; Cl, 29·71 per cent).

Action of Magnesium Methyl Iodide on dl-iso*Nitrosothiocamphor.*—An ethereal solution of *iso*nitrosothiocamphor (15 g.) was slowly added to magnesium methyl iodide (prepared from 2·5 g. of magnesium). The blue solution changed to brown. The mixture was left overnight after which the product was decomposed by means of ice and acetic acid added just to dissolve the precipitated magnesium hydroxide. The solution was then extracted with ether and the ethereal solution dried over anhydrous sodium sulphate. After evaporation of ether, the residue gave on distillation an orange liquid, b. p. 105-106°/6 mm. It is not soluble in water, acids or alkalis, and does not form any lead salt like mercaptans, nor does it decolourise iodine. It is readily soluble in organic solvents like alcohol, ether and chloroform. [Found : C, 67·84 ; H, 8·52 ; N, 7·21 ; S, 16·45. M. W. in benzene (cryoscopic), 195. $C_{11}H_{17}NS$ requires C, 67·69 ; H, 8.72 ; N, 7·18 ; S, 16·41 per cent. M. W., 195).

Action of Methyl Iodide on iso*Nitrosothiocamphor in presence of Sodium Ethoxide.*—Sodium (1·8 g.) was dissolved in alcohol (125 c. c.) and *iso*nitrosothiocamphor (15 g.) added to the solution. To the mixture, methyl iodide (15 g.) was then added. The whole was heated under efficient reflux for 4 hours at 80° when the blue solution turned green. It was then diluted with ice-cold water and extracted with ether. The residue obtained from the ethereal solution gave a blue liquid, b. p. 95-100°/6 mm. The liquid is insoluble in water and in cold alkalis showing the absence of any free : NOH group. [Found : C, 62·71 ; H, 8·12 ; N, 6·72 ; S. 15·18; M. W. cryoscopic (in benzene), 212·5. $C_{11}H_{17}ONS$ requires C, 62·56 ; H, 8·06 ; N, 6·64 ; S, 15·16 per cent. M. W., 211). The blue liquid on hydrolysis with boiling dilute hydrochloric acid (1:1) gave methylmercaptan (isolated as yellow lead salt) and *iso*nitrosocamphor, m. p. 152°.

Hydrolysis of iso*Nitrosothiocamphor.*—A mixture of *iso*nitrosothiocamphor (10 g.), formaldehyde (15 c. c.) and 2 drops of concentrated sulphuric acid was heated on the water-bath till it went into solution.

It was then treated with fuming hydrochloric acid (30 c. c.) and heated for 2 hours. The product was distilled in steam, when a yellow solid passed over which was subjected to fractional crystallisation from 90% alcohol. The yellow prismatic needles obtained in the first fraction melted at 197-98° and were identical with those of camphorquinone. (Found : C, 71·65 ; H, 8·5. Calc. for $C_{10}H_{14}O_2$: C, 72·29 ; H, 8·43 per cent). The mother liquor gave monothiocamphorquinone as greenish yellow needles, m. p. 196°. (Found C, 66·12 ; H, 8·02 : S, 17·69. $C_{10}H_{11}OS$ requires C, 65·93 ; H, 7·7 ; S, 17·58 per cent). Monothiocamphorquinone on treatment with hydroxylamine hydrochloride and sodium hydroxide gave β-camphorquinone dioxime, m. p. 248° with evolution of sulphuretted hydrogen.

The Benzoyl Derivative.—*iso*-Nitrosothiocamphor (10 g.) was taken in benzoyl chloride (8 g.) and a 10 % solution of sodium hydroxide was gradually added to the mixture. After each addition the solution became brown, the colour, however, disappeared on shaking. The separated greenish blue precipitate crystallised from dry ether as bluish green needles, m. p. 115-16°. (Found : N, 4·72 ; S, 10·55. $C_{17}H_{19}O_2NS$ requires N, 4·65 ; S, 10·63 per cent).

Isomeric form of iso*Nitrosothiocamphor* (β-Nitroso-α-thiolbornylene).—After separation of the crystals of *l-iso*nitrosothiocamphor (Sen, *loc. cit.*) the turbid aqueous solution gave out mercaptanic smell. It was extracted with ether, the ethereal solution dried over anhydrous sodium sulphate, ether evaporated and the residual liquid distilled when it passed over as a blue liquid at 105-106°/5 mm. having mercaptanic smell. It is insoluble in mineral acids but dissolves in alkalis giving a yellow solution. It is also soluble in acetic acid and other organic solvents giving bluish green solutions. Owing to the extremely poor yield of the material further study has not been possible. [Found : C, 60·59 ; H, 7·72 ; N, 7·18 ; S, 16·23 per cent. M. W. in benzene (cryoscopic), 195. $C_{10}H_{15}ONS$ requires C, 60·91 ; H, 7·61 ; N, 7·11 ; S, 16·24 per cent. M. W., 197].

My sincere thanks are due to Dr. P. K. Bose for his kind interest in this investigation and also for the facilities provided by him in his laboratory. My thanks are also due to Prof. S. N. Bose of the Dacca University for his valuable help in taking the absorption spectra.

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NEW ASPECTS OF NITROGEN FIXATION AND CONSERVATION IN THE SOIL. PART II.

By N. R. Dhar and S. K. Mukerji.

Experiments have been made on soils after adding organic acids (both high and low carbon), oils like mahua, mustard and linseed and their effect on the fixation of nitrogen have been studied. Effects of canesugar, inulin, starch, cowdung and molasses in the nitrogen fixation in soil both in the artificial light and sunlight as well as in the dark have been investigated. Influence of temperature and light on nitrogen fixation has also been studied and the mechanism of nitrogen fixation with reference to energy change discussed.

We have discovered that sunlight or artificial light markedly increases nitrogen fixation in the soil mixed with energy materials like, carbohydrates, cellulose, fats, etc. Thus when 1 kg. of soil is mixed with definite weight of carbohydrates like canesugar, glucose etc. in basins and exposed to sunlight or artificial light daily for eight hours and another vessel containing the same material is kept in the dark, there is more nitrogen fixation in the vessels receiving light than in the dark.

The moisture is made up to 20% in the beginning to mix the ingredients properly. As there is considerable evaporation, more so in the light than in the dark, distilled water to the extent of 16% per cent. is added daily to the basins exposed to light and after three days in the basins kept in the dark, so as to keep a uniform moisture content. From time to time, the ammoniacal nitrogen, nitric nitrogen, total nitrogen and total carbon are estimated according to the modified Kjeldahl method of Robinson, McLean and Williams (*J. Agric. Sci.*, 1929, **19**, 315). The ammoniacal nitrogen was determined by treating 50 g. of the air-dried soil with 5 g. of pure potassium chloride and 5 g. of pure magnesium oxide and 100 c.c. of water and distilled for six hours upon a water-bath. A current of air, purified by passing through a solution of ferrous sulphate and sulphuric acid, was aspirated, and the ammonia produced was absorbed in dilute sulphuric acid and estimated colorimetrically by nesselerisation in a Duboscq colorimeter. The nitric nitrogen was estimated by treating the soil from which ammonia has been removed by the previous procedure with 1 g. of Devarda's alloy free from ammonia or nitrate and 25 c.c. or 1 % sodium hydroxide solution and left overnight for the reduction of nitrite and nitrate to ammonia, which was estimated as in the previous case. The following are the experimental results.

Nitrogen Fixation with Organic Acids in Sunlight.

The soil (500 g.) was treated with 25 g. of pure acetic, oxalic, tartaric, critic, oleic, stearic, and palmitic acid. Very little fixation was observed with acetic and oxalic acids; with tartaric and citric acids, the increase in the total nitrogen of the soil was by a few mg. only, whereas with stearic, palmitic and oleic acids, a considerable amount of nitrogen fixation took place in one and half a year. Figures in the following tables indicate in mg. the amount of nitrogen fixed per 100 g. of soil.

TABLE I.

Stearic acid.

Started on 25-8-36.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of analysis.
1·0	2·2	56·0	5152	4-9-36
1·2	2·0	56·0	5044	18-11-36
1·2	2·0	56·0	4923	9-1-37
0·6	2·2	55·4	3963·4	20-8-37
1·6	2·0	72·6	2572·5	24-1-38

Palmitic acid.

Started on 25-8-36.

1·0	2·2	56·0	6159	4-9-36
1·0	2·0	56·0	6145	18-11-36
1·2	2·0	58·0	6001	9-1-37
0·2	1·8	57·6	5864·2	20-8-37
1·2	1·8	66·7	2790	24-1-38

Oleic acid.

Started on 25-8-36.

1·0	2·2	56·0	5182·7	4-9-36
1·0	2·0	56·0	5086·7	18-11-36
1·3	2·1	56·0	5000	9-1-37
0·6	2·2	55·8	4123·4	20-8-37
1·4	1·7	63·6	1684·4	24-1-38

Nitrogen Fixation with Oils in Sunlight.

TABLE II.

500 G. soil + 50 g. linseed oil + 5 g. $CaCO_3$.

Started on 25-11-36.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of analysis.
1·2	1·6	58·3	6288	29-8-36
1·4	1·8	58·3	5991	11-9-36
1·4	1·8	58·3	5922	19-10-36
Traces	1·0	58·3	5912	7-1-37
Traces	0·6	58·3	5864	14-4-37
1·1	1·4	87·4	3530	15-1-38
	500 G. soil + 50 g. mustard oil + 5 g. $CaCO_3$.			
1·4	1·8	51·1	6159	29-8-36
1.6	2·2	58·3	6000	11-9-26
1·6	2·2	58·3	6000	19-10-36
Traces	0·8	58·8	5981	7-1-37
Traces	0·6	58·8	5786	14-4-37
1·2	1·6	77·8	3900	15-1-38
	500 G. soil + 50 g. mahua oil + 5 g. $CaCO_3$.			
1·2	1·6	63·6	6947	29-8-36
1·4	1·9	66·7	6428	11-9-36
1·4	1·9	66·7	6411	19-10-36
Traces	1·1	66·7	6412	7-1-37
Traces	0·6	66·7	6321	14-4-37
1·8	1·8	93·4	3600	15-1-38

Other energy materials have also been utilised (*cf. Proc. Nat. Inst. Sci. India*, 1937, **3**, 51, and Presidential address, Nat. Acad. Sci. India, 1937).

We have also investigated the same phenomenon in artificial light. A 1,000 watt gas-filled tungsten filament lamp was placed at a distance of 30 cm. above the basins containing the soil and energy materials. In these experiments the other conditions were the same as in the experiments in the sunlight. The following results have been obtained. Figures in the tables indicate the amount of nitrogen fixed in mg./100 g. soil.

Experiments in Artificial light with Canesugar.

TABLE III.

200 G. of soil + 2 g. of canesugar.

Started on 23-1-37.

Exposed to *light* for 8 hours daily.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
				original soil
1·6	2·4	45·1	499·8	4-2-37
1·7	2·4	48·2	650·5	3-3-37
2·6	2·4	54·4	544·0	3-3-37
2·4	2·5	60·9	418·5	12-4-37
2·6	2·6	59·2	418·7	12-8-37

Nitrogen fixed per g. of carbon oxidised = 33 mg.

Dark.

1·6	2·4	45·1	499·8	Original soil
1·5	2·4	45·1	739·3	4-2-37
1·4	2·4	46·6	636·1	3-3-37
1·7	2·4	48·2	401·7	12-4-37
2·4	2·6	48·2	401·7	12-8-37

Nitrogen fixed per g. of carbon oxidised = 6·2 mg.

200 G. of soil + 4 g. of canesugar.

Exposed to *light* for 8 hours daily.

1·6	2·4	45·1	499·8	Original soil
1·8	2·4	51·1	837·0	4-2-37
2·9	2·4	56·9	661·2	3-3-37
2·2	2·6	63·6	501·2	12-4-37
2·6	2·7	62·8	444·8	12-8-37

Nitrogen fixed per g. of carbon oxidised = 23 mg.

TABLE III (*contd.*).

Dark.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
1·6	2·4	45·1	499·8	Original soil
1·6	2·4	46·7	979·2	4-2-37
1·3	2·4	46·6	712·4	3-3-37
1·6	2·4	51·1	401·7	12-4-37
2·6	2·6	50·1	401·7	12-8-37

Nitrogen fixed per g. of carbon oxidised = 6·8 mg.

200 G. soil + 6 g. canesugar.

Exposed to *light* for 8 hours daily.

1·6	2·4	45·1	499·8	Original soil
1·8	2·4	52·2	1021·1	4-2-37
3·0	2·4	58·3	736·5	3-3-37
2·5	2·8	66·7	527·3	12-4-37
2·6	2·8	65·1	454·8	12-8-37

Nitrogen fixed per g. of carbon oxidised = 20 mg.

Dark.

1·6	2·4	45·1	499·8	Original soil
1·6	2·4	47·6	143·9	4-2-37
1·0	2·4	48·2	837·0	13-3-37
1·6	2·4	52·3	401·7	12-4-37
2·6	2·6	51·8	401·7	12-8-37

Nitrogen fixed per g. carbon oxidised = 5·5 mg.

TABLE III (*contd.*).

200 G. soil + 10 g. canesugar.

Exposed to *light* for 8 hours daily.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
1·6	2·4	45·1	499·8	Original soil
1·8	2·4	51·1	1255·0	4-2-37
3·2	2·4	61·1	837·0	3-3-37
2·6	2·6	73·6	560·7	12-4-37
2·6	2·9	71·2	488·6	12-8-37

Nitrogen fixed per g. of carbon oxidised = 14 mg.

Dark.

1·6	2·4	45·1	499·8	Original soil
1·5	2·4	47·6	2098·0	4-2-37
1·0	2·0	50·0	1807·0	3-3-37
1·6	2·4	56·0	477·0	12-4-37
2·6	2·6	55·2	413·2	12-8-37

Nitrogen fixed per g. carbon oxidised = 5·4 mg.

Experiments in Artificial Light with Inulin.

TABLE IV.

Started on 2-9-37. 200 G. soil + 2 g. inulin. Exposed to *light* daily for 8 hours.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
0·9	3·5	43·7	460·3	Original soil
1·0	1·5	46·7	621·4	18-9-37
1·6	1·6	47·8	611·0	18-10-37
1·2	1·5	58·6	456·5	18-1-38

Nitrogen fixed per g. of carbon oxidised = 30 mg.

TABLE IV (*contd.*).

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
		Dark.		
0·9	3·5	43·7	460·3	Original soil
1·5	1·7	44·2	671·2	18-9-37
1·5	1·7	45·1	661·2	18-10-37
1·2	3·5	51·5	448·8	18-1-38

Nitrogen fixed per g. of carbon oxidised = 17 mg.

200 G. soil + 4 g. inulin. Exposed to *light* daily for 8 hours.

0·9	3·5	43·7	460·3	Original soil
1·2	2·1	48·3	887·2	18-9-37
2·0	1·9	49·3	686·3	18-10-37
3·5	2·0	68·2	568·1	18-1-38

Nitrogen fixed per g. of carbon oxidised = 27 mg.

Dark.

0·9	3·5	43·7	460·3	Original soil
1·6	1·8	45·1	877·4	18-9-37
1·8	1·8	46·8	711·2	18-10-37
1·8	1·3	63·6	652·4	18-1-38

Nitrogen fixed per g. of carbon oxidised = 24 mg.

200 G. soil + 6 g. inulin Exposed to *light* daily for 8 hours.

0·9	3·5	43·7	460·3	Original soil
1·3	1·7	49·3	1915·1	18-9-37
2·4	1·2	51·2	1590·0	18-10-37
1·7	2·5	70·0	712·8	18-1-38

Nitrogen fixed per g. of carbon oxidised = 25 mg.

TABLE IV (*contd.*).

Dark.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
0·9	3·5	43·7	460·3	Original soil
1·6	1·8	46·2	1985·2	18-9-37
2·2	1·8	47·5	1315·0	18-10-37
1·6	1·2	64·8	719·8	18-1-38

Nitrogen fixed per g. of carbon oxidised = 20 mg.

200 G. soil + 10 g. inulin. Exposed to *light* daily for 8 hours.

0·9	3·5	43·7	460·3	Original soil
1·1	1·4	50·0	3296·0	18-9-37
2·8	1·6	52·0	2219·0	18-10-37
1·4	2·8	77·8	887·2	18-1-38

Nitrogen fixed per g. of carbon oxidised = 18 mg.

Dark.

0·9	3·5	43·7	460·3	Original soil
1·6	2·0	47·5	3232·0	18-9-37
1·6	1·8	48·2	1986·0	18-10-37
1·6	1·2	66·7	962·5	15-1-38

Nitrogen fixed per g. of carbon oxidised = 13 mg.

Influence of Temperature on Nitrogen Fixation.

In order to investigate the influence of temperature on nitrogen fixation in the dark, experiments were carried on in dishes in four replications placed in incubators kept in the dark at 20°, 30°, 35°, 40°, 45° and 50°. One set of basins was also exposed to sunlight to obtain comparable results in light. The following results have been obtained at different temperatures.

2% Glucose used in these experimenis and the figures represent the amounts of total nitrogen in 100 g. of soil in mg.

TABLE V.

I.	II.	III.	IV.	V.	VI.	VII.	
20°	30°	40°	45°	50°	35°	Light	
38·8	41·6	41·1	35·0	35·0	43·7	45·1	1
38·0	41·2	40·5	34·8	34·1	42·5	46·4	2
38·5	42·8	40·8	34·5	33·4	43·0	46·7	3
37·8	42·0	40·0	35·5	33·9	43·5	43·7	4

Original soil contained 33·7 mg. of total nitrogen.

The amount of nitrogen fixed in different cases.

	I.	II.	III.	IV.	V.	VI.	VII.
	20°	30°	40°	45°	50°	35°	Light
	5·1	7·9	7·4	1·3	1·3	10·0	11·4
	4·3	7·5	6·8	1·1	0·4	8·8	12·7
	4·8	9·1	7·1	0·8	0·3	9·3	13·0
	4·1	8·3	6·3	1·8	0·2	9·8	10·0
Total	18·3	32·3	27·6	5·0	1·6	37·9	47·1
Mean	4·57	8·2	6·9	1·25	0·4	9·42	11·77

The above experiments show that the nitrogen fixation in the dark is highest at 35° and fails off with either increase or decrease of temperature. The nitrogen fixation in light, is, however, greater than the highest obtained in the dark.

Using completely sterile soil and mixing with energy materials nitrogen fixation has also been observed, 100 grams of ordinary garden soil were mixed with the requisite quantity of the energy material and distilled water and was placed in an autoclave in a silica or glass flask plugged with cotton wool for three to four hours for sterilising the mixture, which was then either exposed or kept in the dark. The following results have been obtained.

TABLE VI.

Exposed to sunlight from 8-5-37 to 15-7-37.

(Figures indicate in mg./100 g. of soil).

Condition.	NH_3-N.	NO_3-N.	Total N.	Total C.	*N* fixed per g. of C oxidised.
Original soil	0·5	1·6	42·4	435·2	
anesugar 2% in a quartz flask	1·2	2·3	43·7	891·4	3·8 mg.
Canesugar 5% in a quartz flask	0·9	1·5	45·1	1740·9	4
Inulin 2% in a quartz boiling tube	1·1	1·7	48·3	444·1	5·9
Inulin 5% in a quartz boiling tube	1·0	2·1	49·8	1246·5	6·2
Starch 2% in a quartz flask	1·3	1·6	46·7	452·1	5·5
Starch 5% in a quartz flask	8·0	2·9	47·1	562·8	2·5

Dark.

The vessels were kept in the same place wrapped with black cloth.

Canesugar 2%	1·1	1·6	42·4	962·6	Nil
Canesugar 5%	0·9	1·6	42·4	1021·4	
Inulin 2%	0·9	1·6	43·7	535·6	1·4 mg.
Inulin 5%	0·7	1·6	42·1	1268·4	
Starch 2%	1·1	1·6	42·4	540·3	Nil
Starch 5%	0·6	1·6	42·4	987·6	

Exposed to sunlight from 25th Sept., 1937 to 2nd March, 1938.

Original soil	36·8	496·9	
Canesugar 2·5% in a 500 c.c. Jena conical flask	38·9	1240·4	4·1 mg.
Glucose 2·5% in a 500 c.c. Jena conical flask	39·8	1180·1	4·5

Dark.

Canesugar 2·5% in a 500 c.c. Jena conical flask wrapped with black cloth and kept by the side of the above flasks	37·2	1312·0	0·9
Glucose 2·5% in a 500 c.c. Jena conical flask as above	37·8	1258·0	2·0

The foregoing results show that under comparable conditions, the amount of nitrogen fixed per gram of carbon oxidised is always much greater in light than in the dark. Moreover our experimental results show definitely that the increased nitrogen fixation observed in the light is not due to increase of temperature when the mixture is exposed to light. It has been observed that the optimum temperature for nitrogen fixation is 35° in the dark; when the temperature is increased or decreased, the nitrogen fixation becomes less. The temperature of the basins receiving sunlight or artifical light is appreciably higher than the optimum although the fixation in light is always greater than even at the optimum temperature in the dark. Another remarkable behaviour is also observed that although azotobacter numbers in the exposed dishes or in fields is much smaller than in the covered ones, the amounts of nitrogen fixed are always greater in light than in the dark (*cf.* Presidential Address National Acad. Sci. India, Jan. 1937). In the basin experiments, the azotobacter in the soils kept in the dark are approximately ten times greater in the dark than in the sunlight, but the nitrogen fixation in basin kept in light is more than double than in those kept in the dark. In the field trials, also the same behaviour is observed, although the azotobacter numbers are smaller in the plots receiving sunshine than in the covered ones, the nitrogen fixation is also double in light than in the dark. In experiments completely anærobic conditions are maintained by stirring the sol and if nitrogen fixation in the soil is entirely a bacterial process, the nitrogen fixation should have been proportional to the azotobacter numbers, but it is otherwise.

We have repeatediy observed that the maximum error possible in our basin experiments in the estimation of total nitrogen never exceeds 1%. In the field trials, difference in sampling causes a variation of about 3% in the total nitrogen. It is quite clear, therefore, that the largely increased nitrogen fixation observed either in sunlight or in artificial light cannot be ascribed to experimental error.

Moreover, the experimental results recorded with sterile soil show that there is an appreciable amount of nitrogen fixation even in the complete absence of bacteria. This is a new finding to which we attach great importance because nitrogen fixation has been ascribed so far to the growth of micro-organisms but our experiments show that these agents are not absolutely necessary.

Our results show that in no case even in the presence of cowdung and hay containing appreciable amounts of nitrogen in the beginning, the oxidation of carbon in the dark is greater than in light, although the

microbial population is greater in the dark than in light. It has been stated by soil bacteriologists that the velocity of the decomposition of carbohydrates and celluiose materials depends on the presence of available nitrogen in the system The available nitrogen is believed to supply the need of the micro-organisms to build their tissues and the food is supplied by the energy materials. It is believed, therefore, that the decomposition of the energy materials is thus facilitated by the increase of the microbial population. In light, however, no such behaviour is observed, because the velocity of the cellulose or carbohydrate oxidation and the nitrogen fixation in light is greater than that in the dark. It is clear, therefore, that light plays a prominent part in the oxidation of energy materials and nitrogen fixation in the soil.

In order to investigate the maximum amounts of nitrogen fixation possible on the application of molasses to soil the following experiments were performed. Figures in the following tables indicate the amount of nitrogen fixed in mg. per 100 g. of soil.

TABLE VII.

500 G. of soil+125 g. of molasses.

Started on 31-8-36.

	NH_3-N.	NO_3-N.	Total N.	Total C.
Original soil	1·4	2·4	56·0	606·7
19-10-36	3·5	4·6	140·0	3348·0
6-1-37	2·1	5·8	149·9	2511
17-3-37	1·8	5·9	116·7	2199
28-8-37	1·9	10·4	78·6	1386
15-1-38	1·8	2·5	87·5	937·3

500 G. of soil+125 g. of molasses+25 g. of $CaCO_3$.

Original soil	1·4	2·4	56·0	606·7
19-10-36	1·8	3·7	100·0	2080
6-1-37	1·9	3·7	91·2	1674
17-3-37	2·5	3·7	87·5	1227
28-8-37	2·2	6·4	61·2	986·6
15-1-38	1·5	2·1	73·6	700·2

TABLE VIII.

Fixation with cowdung alone.

Started on 16-2-37.

	Light.		*Dark.*	
	Total N.	Total C.	Total N.	Total C.
Original soil	2000	39300	2000·0	39300
20-4-37	1783·6	38500	1986·0	37340
26-8-37	2410·0	34500	2000·0	38400
7-2-38	2169·0	26700	2000·0	30410
14-5-38	2156·0	25840	2000·0	28450

Transfer of Energy from one Chemical change to another and the Mechanism of Nitrogen Fixation.

For over twenty-five years in numerous publications from this laboratory, we have been investigating the initiation of exothermal reactions by the energy obtained from another exothermal reaction. Thus we have shown that the energy derived from the oxidation of an aqueous solution of oxalic acid by potassium permanganate, can form a large amount of calomel from a mixture of mercuric chloride and oxalic acid even in the dark. Moreover, the oxidation of sodium sulphite or ferrous hydroxide or cerous hydroxide or manganons hydroxide by air leads to the oxidation of nickelous hydroxide or carbohydrates, fats, proteins, etc., even in the absence of light. The energy materials like carbohydrates, fats and proteins have also been oxidised simply by passing air through their solutions or suspensions exposed to light. In all these, exothermic reactions have been initiated either through the agency of light or the energy available from another exothermal chemical reaction.

Our results show that nitrogen fixation in sunlight or artificial light is much greater than that obtained in the dark per gram of the energy material oxidised. As the amount of energy available from the oxidation of one gram of energy material is the same, both in the light and the dark, it is

expected that the nitrogen fixation should be the same in light as in the dark, but the experimental results show that with all the energy materials, the nitrogen fixation for the same amount of energy output from the oxidation of the energy material, in light is much greater than in the dark. Hence we are led to conclude that in nitrogen fixation, which is an endothermal reaction, the radiations of the sun or other sources are actually utilised in effecting more nitrogen fixation as in photosynthesis or carbon assimilation in plants or in the formation of ozone by light absorption.

From the foregoing observations it is clear that nitrogen fixation can take place in the dark when the soil is supplied with energy obtained from the oxidation of energy materials, like carbohydrates, pentosans, cellulose, oils, fats, etc., but the nitrogen fixation is considerably increased when these systems also receive sunlight or artificial light, which is utilised in this process as in photosynthesis in the plant kingdom or in the formation of ozone by light absorption.

Recent experiments of Dhar and Seshacharyulu show that the nitrogen fixation in soil fed with 2% glucose in the dark is 7·76 mg., whereas 13·1 mg. of nitrogen are fixed in light per gram of carbon oxidised at a temperature of 42°. Hence light and not increase of temperature is responsible for increased nitrogen fixation in light.

Dhar and Atma Ram have shown that the amount of formaldehyde formed by exposing potassium bicarbonate solutions to sunlight in quartz vessels is smaller than the amount formed in the photo-oxidation of organic substances. Moreover, when we compare the amounts of formaldehyde formed with the number of molecules of bicarbonate decomposed or the organic substance oxidised, a great difference is at once observed. Although the salts of the fatty acids are oxidised to a smaller extent than the carbohydrates and the amino-acids under comparable conditions, the amount of formaldehyde produced is greater in the case of the salts of the fatty acids than with carbohydrates and amino-acids.

It is well known that large amounts of energy are liberated in the oxidation of carbohydrates

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 676 \text{ Cal.}$$

This energy can be utilised in bringing about the endothermal reaction between nitrogen and oxygen ($N_2 + O_2 + 432$ Cal $2NO$) on the soil surface even in the absence of sunlight. The oxide of nitrogen in contact with the

soil moisture and lime can cause the formation of the calcium nitrite and nitrate, which may be reduced to ammonium salts by the excess of carbohydrates added to the soil. Moreover, the energy liberated in the oxidation of carbohydrates on the soil surface may lead to the decomposition of water molecules according to the following equation

$$H_2O \longrightarrow H + OH = 112 \text{ Cal.}$$

The atomic hydrogen can readily combine with molecular nitrogen forming ammonium salts on the soil surface. When soil. mixed with carbohydrates, glycerol, etc., comes in contact with air, the carbon decreases and the total and the ammoniacal nitrogen increases by nitrogen fixation. By light absorption. all these processes, which can take place in the dark provided the energy of the oxidation of the carbohydrates is available on the soil surface, are accelerated and hence more nitrogen fixation per gram of the energy material oxidised is observed in light. Under natural conditions large amounts of nitrogen are fixed in the soil with the energy of the oxidation of cellulose aided by sunlight and this is a phenomenon of great importance to plant life.

According to Moore (1921), sunlight causes a slight union of nitrogen and oxygen in the air resulting in the formation of oxides of nitrogen. Dhar and Sanyal (1925) have observed the formation of traces of nitrous acid when air, freed from impurities, is bubbled through conductivity water in presence of ultraviolet light. This reaction cannot go far, as nitrous acid decomposes readily in light according to the equation

$$3HNO_2 \longrightarrow HNO_3 + 2NO + H_2O.$$

Moreover, nitric acid and nitrates also decompose in light. Similarly the following reaction

$$CO_2 + H_2O + 112 \text{ Cal} \longrightarrow H{\cdot}CHO + O_2$$

can take place to a very limited extent in air as formaldehyde readily decomposes in light. Dhar and Atma Ram obtained 0·001 g. formaldehyde per litre of freshly collected rain water. The combination of nitrogen and oxygen and carbon dioxide and water vapour are greatly accelerated when, along with sunlight, the energy of carbohydrate oxidation is available to the system.

Berthelot (*Compt. rend.*, 1900, **130**, 1345, 1430, 1662) has reported the formation of small quantities of nitric acid in the oxidation of substances like carbon, sulphur, metals and hydrogen especially in his calorimetric bombs At the ordinary pressure even less than 1 mg. of nitric acid is formed with a mixture of nitrogen and oxygen in the oxidation of amorphous carbon. With air the yield of nitric acid is one tenth (Russell, *J. Chem.*

Soc., 1903, **83**, 1263) obtained small quantities of ammonium of nitrite and nitrate in the oxidation of phosphorus by oxygen. It appears that the energy of oxidation can be utilised in nitrogen fixation.

The efficiency of nitrogen fixation is also as low as in the case of carbon assimilation. It is well known that in many cases, only 0·1 to 0·2% of the light energy is utilised in carbon assimilation in plants. In our experiments with nitrogen fixation, not even 1% of the energy liberated by the oxidation of the carbohydrates or cellulosic materials is utilised in nitrogen fixation.

As the soil is porous and the surface is renewed in cultivation, the influence of light can be effective in the soil brought under cultivation. Moreover, the light penetration in soils under tropical conditions is likely to be important.

It is well known that nitrogen is an elusive substance and as much as 60% of the added nitrogenous compound used as manure is lost as gaseous nitrogen from the soil in the course of their oxidation. Hence the nitrogenous compounds formed by fixation are also oxidised on the soil surface and undergo loss due to the formation and decomposition of the unstable substance, ammonium nitrite produced in the course of the oxidation of the proteins ammonium salts and other nitrogenous compounds. The whole of the nitrogen fixed in soil cannot remain there for a long time because of this loss.

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ON THE PHYSICO-CHEMICAL PROPERTIES OF INDIAN BENTONITES. PART I.

By N. C. Sen-Gupta.

The swelling and thixotropy of seven natural Indian bentonites in relation to their chemical composition, exchangeable bases, and base binding capacity have been studied.

Bentonite is being increasingly used in the preparation of muds for rotary drilling. When added in small quantities to a clay suspension, bentonite stabilises the mud and induces thixotropy in it. Freundlich, Schmidt and Lindau (*Koll. Chem. Beih.*, 1932, **36**, 43) have studied the influence of various alkalis and salts on the thixotropy and allied properties of a hydrogen bentonite, prepared from the naturally occurring sodium bentonite of Wyoming (U.S.A.). Bentonite deposits in different countries have variable properties and the need for investigations on Indian clay minerals, similar to bentonite, has been pointed out by Mitra and Reid (*Proc. Indian Science Congress*, 1938, p. 99).

In the present investigation bentonite samples* from different localities of India were taken ; they are named according to the localities of their occurrence :—

Kashmir—KB, Akli—AB, Hatikidhani—HB, Bhadres—BB, Maober—MB, Dodia Bisale—DB. Nimli Nadi—NB.

The chemical composition of bentonites approximates to that of the montmorillonite type of clay. Ross and Shannon (*J. Amer. Cerm. Soc.*, 1926, **9**, 77) propose the formula Al_2O_3, CaO, $5SiO_2$ and Marshall (*J. Phys. Chem.*, 1937, **41**, 935) represents it as Al_2O_3, MgO, $5SiO_2$, mH_2O, while Hofmann *et al* (*Z. Krist.*, 1933, **86**, 340) believe that the fundamental structure is Al_2O_3, $4SiO_2$, nH_2O (prophyllite).

Bentonites possess a layer lattice structure as shown by *X*-ray investigations (Hofmann *et al*, *loc. cit.*). Polar liquids like water can enter in between the layers forcing them appart. Several special properties of bentonites namely thixotropy, high swelling and large sedimentation volume are now ascribed to this property.

The maximum distance to which the layers can be separated by means of water, however, depends on the saturating exchangeable cation. Marshall

* The bentonite samples have been kindly supplied by the Assam Oil Compan , Ltd.

(*loc. cit.*) has calculated that the mean separation in the case of H- or Ca-bentonite is 14Å, while in the case of Na- bentonite it is greater than 23Å. The thixotropic property of bentonite is also destroyed when exchangeable sodium is replaced by hydrogen or calcium.

The following properties of several Indian bentonites have been dealt with in the present paper :—

(*a*) Chemical composition.
(*b*) Base saturation capacity and exchangeable cations.
(*c*) Swelling in distilled water.
(*d*) Sedimentation volume and sedimentation velocity in distilled water.
(*e*) Thixotropy in distilled water.

A comparative study of the influence of saturating cations on the properties of the bentonites has been undertaken.

Experimental.

The samples were all powdered and sieved through a 70 mesh sieve. Chemical analyses were carried out by the sodium carbonate fusion method (Wright, " Soil Analysis," 1934, p. 153). The results are experessed as the percentage composition by weight.

Table I.

Sample.	SiO_2.	Al_2O_3.	Fe_2O_3.	CaO.	MgO.	$Na_2O + K_2O$ (by diff.).	Loss at 110°.	Loss on ignition.
KB	52·4	17·0	1·8	1·9	1·3	2·5	10·3	12·8
AB	54·9	19·4	6·8	0·1	0·0	2·1	10·9	5·8
HB	51·4	21·4	6·8	0·7	1·9	3·1	8·7	6·0
BB	52·4	24·1	6·6	0·4	0·4	0·7	8·1	8·3
MB	48·3	22·7	9·3	0·7	0·3	1·3	7·7	9·7
DB	51·4	24·7	5·2	0·6	1·5	2·2	7·4	7·0
NB	52·1	28·2	5·0	0·4	0·3	1·6	4·1	8·3

The base saturation capacity has been measured by Parker's method (*J. Amer. Soc. Agron.*, 1929, **21**, 1030) and by Schollenberger's method (*Science*, 1927, **65**, 552) and the individual bases by Schollenberger's method only.* In Table II, the results of base exchange measurements are compared with the SiO_2/Al_2O_3 ratio of the various bentonites. The exchangeable bases and the base saturation capacity were measured at p_H 7, and are expressed in milli-equivalents per 100 g. of bentonite dried at 110°.

* The minerals do not contain $CaCO_3$ and the amount of exchangeable Ca as obtained by leaching with sodium chloride (Wright, *loc. cit.*) was practically the same as obtained by Schollenberger's method.

TABLE II.

Sample.	$\frac{SiO_2}{Al_2O_3}$	p_H of 5% suspension.	Base sat. capacity.	Exchangeable Ca.	Mg.	Na + K.	Total exchangeable bases.
KB	5·0	6·8	100	66	1	28	95
AB	4·8	7·2	92	5	0	85	90
HB	4·0	7·1	80	13	19	44	76
BB	4·0	7·1	74	11	15	39	65
MB	3·7	7·1	81	15	16	45	76
DB	3·5	7·4	65	11	9	42	62
NB	3·2	6·7	64	9	9	40	58

Thus all the bentonites are more or less saturated with cations. KB is a calcium bentonite and is expected to have some unusual properties from the rest. AB contains mainly sodium and very small amount of Ca, the rest containing all the replaceable cations in various proportions. The base saturation capacity increases with the SiO_2/Al_2O_3 ratio.

Swelling.

By the term swelling is meant the expansion of the pore-space under the influence of a solvent. In order to get the true swelling of a dry gel one has to subtract the volume of the original pore-space from the total volume of liquid taken in when the swelling substance is in contact with it.

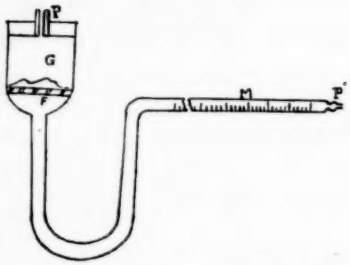
Swelling is a phenomena comparable to osmosis and as such swelling can be measured by the pressure developed on the side of the swelling substance separated by a membrane through which the solid cannot pass.

Since swelling pressure is small for bentonites Freundlich *et al* (*loc. cit.*) devised an apparatus for measuring the volume of liquid taken in instead of pressure. If the original pore-space of the material is known the amount of swelling can be easily obtained. For bentonities the swelling is generally several times greater than the pore-space and the total sorption of water may be taken as proportional to swelling for comparative studies.

The swelling has been measured by a simplified form of the apparatus used by Freundlich *et al* (*loc. cit.*). In Fig. 1 G is a glass gooch in

which the substance is placed on the filter-bed F, M is a capillary burette reading directly up to 0·01 c.c.

Fig. 1



Evaporation is prevented by means of a rubber stopper S and the capillaries P P.' The apparatus is filled upto F and the end of the microburette with distilled water. One gram of the sieved samples of the bentonite dried at 110° is quickly dropped in and the stopper is closed. The rate of absorp tion of water in c.c. is measured from the movement of the water meniscus in the burette.*

TABLE III.

Time.	KB.	AB.	HB.	BB.	MB.	DB.	NB.
	Water	absorbed	per g.	of	oven-dried	bentonite	in c c.
30 sec.	1·26	0·48	0·48	0·39	0·48	0·55	0·30
1 min.	1·36	0·59	0·57	0·46	0·57	0·64	0·38
2	1·41	0·75	0·70	0·56	0·70	0·76	0·46
5	1·45	1·07	0·94	0·76	0·93	0·98	0·62
10	1·48	1·42	1·22	1·00	1·21	1·25	0·77
20	1·50	1·90	1·55	1·31	1·53	1·58	0·95
40	1·52	2·57	1·91	1·67	1·88	2·00	1·09
60	1·53	3·02	2·04	1·81	2·01	2·17	1·145
100	1·54	3·40	2·10	1·94	2·06	2·30	1·195
180	1·56	3·72	2·14	2·02	2·10	2·40	1·24
Final value after 2 days	1·64	5·01	2·52	2·20	2·49	2·79	1·57

* Baver and Winterkorn (*Soil Science*, 1934, **38**, 291) have also measured swelling by the same method.

The results with AB are plotted in Fig. 2, where the curve obtained by Freundlich and co-workers (*loc. cit.*) on natural Wyoming bentonite is given for comparison.*

The high swelling of AB is obviously due to its high percentage of univalent cations. KB shows a swelling curve different from the rest in that the swelling is almost complete in 10 minutes. This is to be ascribed to the high percentage of exchangeable calcium. The swelling of KB is comparable to the case of electrodialysed bentonite studied by Freundlich *et al* (*loc. cit.*).

FIG. 2.

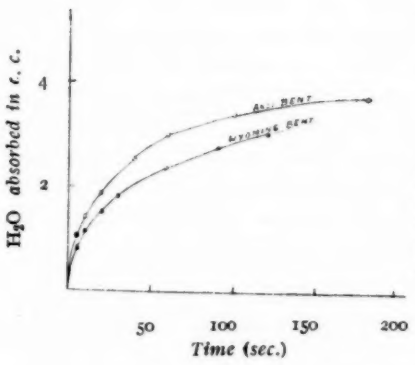
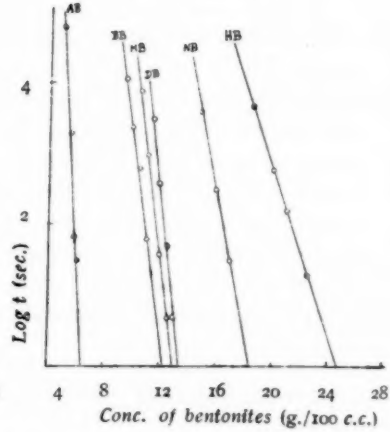


FIG. 3.



Sedimentation Volume and Sedimentation Velocity.

The rate of sedimentation was measured in 10 c. c. cylinders graduated to 0·1 c. c. The results with 5% bentonites dried at 100° are given in Table IV.

TABLE IV.

Sample.	KB.	HB.	BB.	MB.	DB.	NB.
	Sedimentation volume in c.c. at the time *t*					
Time (*t*)						
30 mins.	1·2	8·1	9·1	9·1	9·3	10·0
1 hr.	1·3	7·0	8·6	8·6	8·8	10·0
2	1·4	6·3	8·3	8·2	8·1	10·0
3	1·4	6·1	8·1	8·0	7·8	9·95
5	1·45	5·9	7·9	7·8	7·5	9·95
10	1·55	5·85	7·65	7·5	7·0	9·9
20	1·6	5·8	7·5	7·3	6·7	9·85
Final constant value	1·7	5·7	7·4	7·2	6·6	6·0

* Freundlich *et al* made measurements with 0·3 g. of the sample; their results are divided by 0·3 to give the swelling for 1. g. of the bentonite.

The bentonite AB was thixotropic at this concentration and KB showed differential settling with a turbidity in the upper layer. Excepting NB the sedimentation volume reaches a limiting value practically after one day. The sedimentation volume of NB diminished almost linearly with time for about a week.

Thixotropy.

The time for sol to gel transformation is known to vary largely with the concentration of the suspension, the cross-section of the measuring apparatus and the temperature (Freundlich, "Thixotropy," Paris, 1935).

Definite weights of the powdered and sieved samples were added to 2 c. c. of distilled water in measuring cylinders of the same cross-section (1.14 sq. cm). The time of solidification was measured after the samples had been in contact with the liquid for one day by which equilibrium between them may be expected to have been established. The time of solidification was not satisfactorily reproducible but since it varies exponentially with the solid concentration, an approximate value of the former is sufficient for comparative measurements. The logarithm of the average time has been plotted in Fig. 3 against the percentage concentration of the undried bentonite (drying often reduces thixotropic properties). A linear relationship is observed.

Assuming that the suspensions are thixotropic between setting times 1 sec and 24 hours, the range of thioxotropic concentration are given in Table V.

TABLE V.

Bentonite (air dried) per 100 c. c. of water showing thixotropy.

Bentonite.	Min.	Max.
AB	5·0	6·5
HB	16·5	24·5
BB	9·1	12·2
MB	9·8	13·0
DB	10·7	13·5
NB	13·7	18·4

KB shows gelation by differential settling

Besides the time of gelation one must also know the rate of change of viscosity during the time of gelation and the gel strength before a complete picture of the thixotropic properties of a material can be formed. Further measurements in this line are in progress.

My best thanks are due to Prof. J. N. Mukherjee, D.Sc., for his suggestions and kind interest in the work and to the Calcutta University for granting laboratory facilities. My best thanks are also due to the Assam Oil Company Ltd., for awarding a research scholarship and for the kind permission to publish this work.

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ENZYMES IN SNAKE VENOM. PART IV.

By B. N. Ghosh and D. K. Chowdhury.

The critical inactivation temperature of the proteolytic enzymes in the venoms of cobra (*Naja naja*), banded krait (*B. Fasciatas*), *Echis carinata* and *Vipera russelli* has been determined and has been found to be 55°, 53°, 55° and 62° respectively. The critical inactivation temperature of trypsin mixed with heated cobra venom is 60°. It has therefore been suggested that the proteolytic enzyme in snake venom is identical with trypsin.

In a number of papers published by Ghosh and co-workers (*J. Indian Chem. Soc.*, 1936, **13**, 450, 627 ; 1937, **14**, 604) it has been shown that the venoms of cobra (*Naja naja*), Daboa (*Vipera russelli*), banded krait (*B. Fasciatus*) and *Echis carinata* contain a proteolytic enzyme which resembles trypsin in almost all the properties investigated so far. One of the characteristic properties of enzymes is their critical inactivation temperature, a temperature at which an enzyme loses one half of its original activity when heated for one hour. Although this temperature varies somewhat with the protein content of an enzyme preparation yet it is of much help in establishing the identity of an enzyme. In this paper, the results which have so far been obtained on the determination of the critical inactivation temperature of the proteinase present in the venoms of cobra, banded krait, Russell's viper and *Echis carinata* along with some results on the effect of heated and ordinary venom on the activity of trypsin, is recorded. It will be noted from these results that the temperature of critical inactivation of the proteinase contained in the different venoms, is quite close to that of trypsin admixed with proteins of cobra venom.

Experimental.

Determination of the Critical Inactivation Temperature.

A series of solution of a venom were prepared at different temperatures by dissolving it in water heated to the desired temperature. The solutions were maintained for one hour at the respective temperatures at which they were prepared. They were then quickly cooled under a tap and a fixed quantity (2 c.c.) was withdrawn from each solution and allowed to act separately on 18 c.c. of a 1·9% solution of casein mixed with phosphate buffer at p_H 7·2 and maintnined at a temperatnre of 36° in a thermostat. The extent of hydrolysis was determined by withdrawing at suitable intervals of time 5 c.c. of the solution and titrating it by Sörensen's method (Formol titration). The results are recorded in the following tables. The concentrtion of the substrate (casein) was throughout 1·7%.

TABLE I.

Russell's viper venom, 0·2% solution. Critical inactivation temp., 62°.

Venom heated for 1 hour at	0·01*N*-Alkali required		Diff. between I and II.
	after 2 hrs. digestion. I.	Control. II.	
36°	7·45 c.c.	5·65 c.c.	1·80 c.c.
50°	7·40	5·65	1·75
60°	6·75	5·65	1·10
62°	6·55	5·65	0·90
65°	6·05	5·65	0·40
70°	5·70	5·65	0·05

TABLE II.

Cobra venom, 0·4% solution. Critical inactivation temp., 53°.

Venom heated for 1 hour at	0·01*N*-Alkali required		Diff. between I and II.
	after 2 hrs. digestion. I.	Control. II.	
36°	7·10 c.c.	6·45 c.c.	0·65 c.c.
50°	6·90	6·45	0·45
55°	6·60	6·45	0·15
60°	6·50	6·45	0·05
56°	6·45	6·45	0·00

TABLE III.

Krait venom, 0·4% solution. Critical inactivation temp., 55°.

The venom heated for 1 hour at	0·01*N*-Alkali required		Diff. between I and II.
	after 2 hrs. digestion. I.	Control. II.	
36°	5·70 c.c.	5·40 c.c.	0·30 c.c.
50°	5·65	5·40	0·25
55°	5·55	5·40	0·15
60°	5·40	5·40	0·00
65°	5·40	5·40	0·00

TABLE IV.

Echis carinata venom, 0·1% soln. Critical inactivation temp., 55°.

The venom heated for 1 hour at	0·01*N*-Alkali required		Diff. between I and II.
	after 2 hrs. digestion. I.	Control. II.	
36°	6·80 c.c.	5·30 c.c.	1·50 c.c.
50°	6·40	5·30	1·10
55°	6·10	5·30	0·80
60°	5·60	5 30	0·30
65°	5·30	5·30	0·00

TABLE V.

Enzyme used – trypsin (E. Merck 0·02% soln.) Critical inactivation temp., 50°.

Enzyme heated for 1 hour at	0·01*N* alkali required		Diff. between I and II.
	after 2 hrs. digestion. I.	Control. II.	
30°	6·80 c.c.	5·45 c.c.	1·35 c.c.
45°	6·35	5·45	0·90
50°	6·15	5·45	0·70
55°	5·55	5·45	0·10

TABLE VI.

Enzyme preparation used – trypsin dissolved in 2% cobra venom solution previously heated to 70°. Substrate used, casein 1·7%. Critical inactivation temperature, 60°.

The enzyme heated for 1 hour at	0·01*N*-Alkali required		Diff. between I and II.
	after 2 hrs. digestion. I	Control. II	
30°	10·65 c.c.	5·85 c c.	4·80 c.c.
36°	10·65	5·85	4·80
50°	10·30	5·85	4·45
55°	9·65	5·85	3·80
60°	8·20	5·85	2·35
65°	6·75	5·85	0·90

It will be noticed from the data recorded in the Tables I-VI, that critical inactivation temperatures of the proteinase in the venoms of cobra, krait. *Echis carinata* and Russell's viper are 53°, 55°, 55°, and 62° respectively, while that of trypsin (E. Merck) is about 50° and that of trypsin dissolved in previously heated Cobra venom solution is 60°. It may be mentioned here that Arrhenius (" Immuno-Chemistry," 1907, p. 90) found 65° to be the critical inactivation temperature of trypsin, considering the fact that the critical inactivation temperature of trypsin may vary from 50° to 60° or to a still higher value depending on the nature and extent of the inert proteins associated with it, it is perhaps permissible to assume that the proteinases in the venoms used are one and the same enzyme, trypsin, but show somewhat different critical inactivation temperatures owing to the different kinds and quantities of proteins with which they are associated in the different venoms.

Effect of Heated and Ordinary Venom on the Activity of Trypsin.

In a previous publication (Ghosh and De, *J. Indian Chem. Soc.*, 1936, **13**, 637) it has been shown that the venoms (not heated) of cobra and Russell's viper inhibit the activity of trypsin (E. Merck) and this has been attributed to the presence of some inhibitor of trypsin in the two venoms used. The effect of heat on the destruction of this inhibitor has been investigated and the results obtained, so far, are recorded in this paper. Solutions containing known amounts of venom were prepared and heated for one hour at temperatures above 70° so that the proteinase present in them is destroyed. 2 C.c. of the heated venom solution were then added to different flasks containing the substrate and other requisite substance. The p_H of the reaction media was adjusted at the desired value and they were buffered with phosphate solution of the same p_H. The volume of the reaction media was 20 c.c. in each case. The concentrations of heated and ordinary venom were the same in all the flasks in which they were added. Similarly, the concentration of trypsin was kept the same in all the flasks in which it was added. The solutions were placed in a thermostat at 26° and the extent of hydrolysis was estimated by withdrawing 5 c.c. of the solution at suitable intervals of time and titrating it by Sörensen's method. The results are recorded in the following tables in which h.v. stands for heated venom, v for ordinary (*i.e.* not heated) venom, T for trypsin, h.v. + T for mixtures of heated venom and trypsin and v + T for mixtures of ordinary venom and trypsin. The control consists of the substrate solution only.

Table VII.

Cobra venom = 0·1 % soln. Substrate used = 1·7% gelatin (Difco) soln.

p_H.	Period of digestion.	No. of c.c. of 0·01*N*-alkali required. h.v.	v.	T.	h v.+T.	v. + T.	Control.	No.
7·1	0 hrs.	4·80	4·80	4·85	4·80	4·85	4·80	1
	3	4·80	5·10	8·50	9·45	7·80	4·80	2
	Diff. bet. 1 & 2	0·00	0·30	3·65	4·95	2·95	0·00	
8·0	0	3·25	3·25	3·20	3·25	3·20	3·20	3
	3	3·25	3·65	6·95	7·90	6·50	3·20	4
	Diff. bet. 3 & 4	0·00	0·40	3·75	4·65	3·30	0·00	

Substrate used = 1·7 % casein solution.

7·1	0	4·45	4·45	4·45	4·45	4·45	4·40	5
	3	4·45	4·70	8·80	9·70	7·40	4·40	6
	Diff. bet. 5 & 6	0·00	0·25	4·35	5·25	2·95	0·00	
8·0	0	4·40	4·40	4·40	4·40	4·40	4·40	7
	3	4·40	4·70	8·45	10·15	7·14	4·40	8
	Diff. bet. 7 & 8	0·00	0·30	4·05	5·75	2·75	0·00	

Table VIII.

Banded krait venom = 0·1 %. Substrate used = 1·7 % gelatin (Difco) soln.

p_H.	Period of digestion.	No. of c.c. of 0·01*N*-alkali required. h.v.	v.	T.	h.v. + T.	v. + T.	Control.	No.
7·1	0 hrs	4·00	4·00	4·00	4·00	4·00	4·00	1
	3	4·00	4·10	7·35	8·20	8·25	4·00	2
	Diff. bet. 1 & 2	0·00	0·10	3·35	4·20	4·25	0·00	
8·0	0	2·20	3·20	3·20	3·20	3·20	3·15	3
	3	3·20	3·40	6·70	7·95	7·95	3·15	4
	Diff. bet. 5 & 4	0·00	0·20	3·50	4·75	4·75	0·00	

TABLE VIII (*contd.*).

p_H	Period of digestion.	No. of c.c. of 0·01 *N*-alkali required. h.v.	v.	T.	h.v. + T.	v. + T.	Control.	No.
		Substrate used = 1·7% casein solution.						
7·1	0 hrs.	4·80	4·80	4·80	4·80	4·80	4·75	5
	3 hrs.	4·80	4·90	9·70	10·20	10·20	4·75	6
Diff. bet. 5 & 6.		0·00	0·10	4·90	5·40	5·40	000	
8·0	0 hrs.	4·40	4·40	4·40	4·40	4·40	4·40	7
	3 hrs.	4·40	4·60	9·00	10·05	9·50	4·40	8
Diff. bet. 7 & 8.		0·00	0·20	4·60	5·65	5·10	0·00	

TABLE IX.

Russell's viper venom = 0·1 % soln. Substrate = 1·7% casein soln.

p_H	Period of digestion.	No of c.c. of 0·01 *N* alkali required. h.v.,	v. + T.		h.v. + T.,	v. + T.,	Control.	No.
7·1	0 hrs.	4·95	4·95	4·95	4·95	4·95	4·90	1
	3 hrs	4·95	5·35	8·80	6·80	7·20	4·90	2
Diff. bet. 1 & 2.		0·00	0·40	3·85	1·85	2·25	0·00	
		Substrate used = gelatin (Difco) solution (1·7%)						
7·1	0 hrs	4·00	4·00	4·00	4·00	4·00	4·00	3
	3 hrs.	4·00	4·40	7·25	4·60	4·85	4·00	4
Diff. bet. 3 & 4		0·00	0.40	3·25	0·60	0·85	0·00	

TABLE X.

Echis carinata venom = 0·1% solution.
Substrate = (Difco) gelatin 1·7% soln.

p_H	Period of digestion	No. of c.c of 0·01 *N*-alkali required. h.v.	v.	T.	h.v. + T.	v. + T.	Control.	No.
7·1	0 hrs.	4·00	4·05	4·05	4·00	4·05	4·05	1
	3 hrs.	4·00	5·65	7·35	8·35	9·05	4·05	2
Diff. bet. 1 & 2.		0·00	1·60	3·30	4·35	5·00	0·00	
8·0	0 hrs.	3·35	3·40	3·35	3·35	3·40	3·35	3
	3 hrs.	3·35	5·25	6·85	7·75	8·50	3·35	4
Diff. bet. 3 & 4.		0·00	1·85	3·50	4·40	5·10	0·00	

TABLE X (*Contd.*).

p_H.	Period of digestion	No. of c.c. of 0·01. *N*-alkali required. h.v.	v.	T.	h.v. + T.	V. + T	Control.	No.
		Substrate used = 1·7% casein solution.						
7·1	0 hrs.	4·95	4·95	4·95	4·95	4·95	4·95	5
	3 hrs.	4·95	6·40	8·95	10·70	11·65	4·95	6
Diff. bet. 5 & 6.		0·00	1·45	4·00	5·75	6·70	0·00	
8·0	0 hrs.	4·35	4·35	4·35	4·35	4·35	4·35	7
	3 hrs.	4·35	5·60	8·15	10·00	10·85	4·35	8
Diff. bet. 7 & 8.		0·00	1·25	3·80	5·65	6·50	0·00	

It will be noticed from the data recorded above that the addition of solutions of venoms of cobra, banded krait and *echis carinata* previously heated at 70° for one hour to trypsin increases its activity markedly. The venoms of krait and *echis carinata*, even when they are not heated, activate trypsin but solutions of cobra venom which is not heated and Russell's viper venom whether heated or not, inhibit the activity of trypsin to an appreciable extent.

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PHOTOTROPY AND PHOTOCHEMICAL ISOMERISM FROM THE MAGNETIC STANDPOINT.

By S. S. Bhatnagar, P. L. Kapur and Mahbub Shah Hashmi.

Magnetic properties of some phototropic and photoisomeric compounds have been investigated. The distinct change in the magnetic susceptibility value of cinnamylidene-malonic acid and thiophosgene observed on exposure to light, has been ascribed to the formation of a polymer and that of *o*-nitrobenzaldehyde to the formation of an isomer. No effect on the magnetic susceptibility value of anils and hydrazones has been observed on exposure to light. On the basis of the magnetic data obtained, the view of Senier and Shepherd, that the change in colour of anils and hydrazones on exposure to light is due to the change in the aggregation, has been favoured, and other theories put forth to account for the mechanism of phototropy have been discussed.

Much speculation is rife as regards the nature of transformations which take place when a substance undergoes a phototropic change. This change is completely or partially reversible when the exposed substances are kept in the dark for a sufficiently long time, heated or recrystallised. Marckwald (*Z. physikal. Chem.*, 1899, **30**, 140) from the phototropic behaviour of α-tetrachloroketonaphthalene concluded that the phenomenon was a purely physical one, bound up with the crystal form of the substance. Senier and Shepherd (*J. Chem. Soc.*, 1909, **95**, 441, 1943), as a result of the study of a large number of anils, hydrazones and fulgides considered the phototropic change simply as being due to 'the existence of complex solid molecular aggregates related to gaseous molecules as gaseous molecules are related to atoms.' It is implied that different aggregates have different colours. Chemical theories suggesting the formation of new compounds on exposure to light have been put forth. But all of these suffer from the handicap that no one has been able to isolate or identify or even conclusively show the existence of the hypothetical new compound formed by light. The authors have undertaken to examine the mechanism of phototropy from the changes in magnetic properties which should take place when either a new compound is formed or when polymerisation takes place in a compound or when there is a change in crystal structure on exposure to light.

Experimental.

Preparation of Compounds.—The anils and hydrazones were prepared by condensing the aldehydes with amines and hydrazines respectively

(Emmerich, *Annalen*, 1887, **241**, 351; Senier and Shepherd, *J. Chem. Soc.*, 1909, **95**, 1943; Biltz, *Annalen*, 1899, **305**, 170). The crude condensed product was recrystallised from suitable solvents till the melting point of the compound prepared was in agreement with that given in literature.

Cinnamylidene-malonic acid was prepared according to the method of Rüber (*Ber.*, 1902, **35**, 2211).

Thiophosgene was prepared according to the method of Frankland and Garner (*J. Soc. Chem. Ind.*, 1920, **39**, 314T). It was kept over calcium chloride and boiled at 75°. It is a red liquid turning to a white crystalline product on exposure to light. The white compound separated and recrystallised from light petroleum melted at 117°. The previously reported m.p. is 116-119° (*Ber.*, 1933, **66**, 567).

o-*Nitrobenzaldehyde*.—Kahlbaum's extra pure compound was recrystallised from benzene.

Maleic Acid.—Kahlbaum's extra pure maleic acid was recrystallised from an aqueous solution and dried at 100°.

All these compounds, after properly drying and testing their purity by comparing their melting points and colour changes on exposure to light with those given in literature, were stored in glass tubes wrapped with black paper and were placed in a vacuum desiccator till further use.

For magnetic investigations, the magnetic susceptibility was determined on the Bhatnagar-Mathur Interference balance (*Phil. Mag.*, 1929, *vii*, **8**, 1041) manufactured by Adam Hilger, Ltd., London and the Gouy's balance. The compound was then exposed to light in a vacuum desiccator. The mass was thoroughly raked at regular intervals in order to enable a uniform colour change to take place in the whole bulk of the compound. The magnetic susceptibility of the coloured modification was then determined. It was kept in darkness or was heated to a suitable temperature to recover its original colour. The magnetic susceptibility of the compound was again determined. These processes were repeated on the same compound at least thrice and the mean of a large number of readings for each modification was taken. It was found in all the cases that the magnetic susceptibility value of the compound after the recovery of the colour of the exposed sample was almost identical with the original one.

The results of such measurements of magnetic susceptibility of the compounds both before and after exposure to light are given in TableI.

TABLE I.

	Name of the compound.	$\chi \times 10^7$. Original.	After exposure.
1.	Thiophosgene	−4·401 (liquid)	−3·914 (solid)
2.	Cinnamylidene-malonic acid	−4·835	−4·140
3.	Maleic acid	−4·283	−4·280
4.	β-Naphthylamine-salicylidene :		
	(*i*) Yellow variety	−5·394	−5·389
	(*ii*) Red variety	−5·394	−5·393
5.	*p*-Aminobenzoic acid-salicylidene	−5·070	−5·070
6.	*p*-Aminobenzoic acid ethyl ester salicylidene	−5·900	−5·903
7.	*m*-Phenylenediamine-salicylidene :		
	(*i*) Needle crystals	−4·195	−4·193
	(*ii*) Plate crystals	−4·782	−4·783
8.	*m*-Toluidine-salicylidene	−4·965	−4·966
9.	*o*-Anisidine-salicylidene	−5·792	−5·790
10.	Benzaldehyde-phenylhydrazone	−6·065	−6·075
11.	Benzaldehyde-*p*-bromophenylhydrazone	−5·465	−5·467
12.	5-Bromosalicyldehyde-diphenylhydrazone	−5·465	−5·467
13.	Benzaldehyde-*p*-tolylhydrazone	−6·342	−6·342
14.	Anisaldehyde-phenylhydrazone	−6·090	−6·088
15.	Cinnamyldehyde-phenylhydrazone	−5·872	−5·872
16.	Cinnamyldehyde-*p*-bromophenylhydrazone	−5·381	−5·380
17.	Cinnamyldehyde-*p*-tolylhydrazone	−6·184	−6·185
18.	Cinnamyldehyde-β-naphthylhydrazone	−4·066	−4·066
19.	Vanillin-β-naphthylhydrazone	−6·011	−6·014
20.	Benzilphenylhydrazone	−6·080	−6·080
21.	Benzil-*o*-tolylhydrazone	−6·353	−6·354
22.	Piperonal-*p*-tolylhydrazone	−5·833	−5·832
23.	Piperonal-β-naphthylhydrazone	−4·962	−4·962

In the case of cinnamylidene-malonic acid it was observed, however, that on exposure to light in the presence of air and moisture at 50-60°, the sample did not give repeatable magnetic susceptibility values. The values observed on several occasions were even higher than those of the unexposed sample. It was, therefore, necessary to expose cinnamylidene-malonic acid in vacuum at low temperatures.

The variation observed in the magnetic susceptibility value of the cinnamylidene-malonic acid observed on exposure to light at 50-60° in presence of air and moisture may be due to the formation of some oxidised products of polymerised compounds. The final magnetic susceptibility value of the exposed sample in vacuum, therefore, may not be the value for the polymerised product alone, but it may be the value at the equilibrium point of the various products formed.

It was found during experimentation that *o*-nitrobenzaldehyde melted when exposed in a desiccator to direct sunlight and a solution of alum had to be employed to cut off the heat rays.

The compound first turned green but after a few hours it became whitish and after a few days it changed completely into a white mass. The compound was raked from time to time and the magnetic susceptibilities determined are shown in Table II.

Solution of *o*-nitrobenzaldehyde in acetone when exposed to light turns green and after some time a white compound begins to separate out of the solution. The magnetic susceptibility in solution at three various stages has been determined and the results are shown in Table III.

TABLE II.

For o-nitrobenzaldehyde.

Time of exposure.	Colour.	$\chi \times 10^7$.
0 hour	Yellow	−4·398
12 hours	Greenish yellow	−4·430
36	Greenish yellow (white)	−4·295
50	Greenish yellow and white crystals	−4·267
96	White and slightly greenish	−4·135
6 days	White	−3·993
8 days		−3·930
	o-Nitrosobenzoic acid	−3·926

TABLE III.

Magnetic susceptibility of o-nitrobenzaldehyde in acetone solution.

Susceptibility of acetone $= -5{\cdot}830 \times 10^{-7}$.

Time of exposure.	Colour.	$\chi \times 10^7$.
0 minutes	10% Solution	$-4{\cdot}401$
10	Green solution	$-4{\cdot}430$
60	White nitrosobenzoic acid started separating out	$-4{\cdot}158$

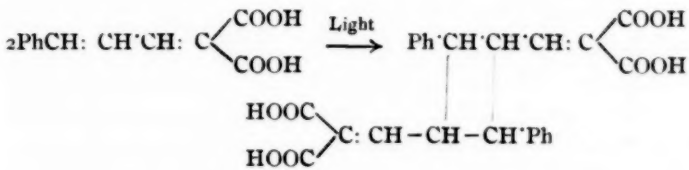
DISCUSSION.

Prominent points which are brought out from the data given above are that on exposure to light :

(*a*) there is a distinct change in the magnetic susceptibility of cinnamylidene-malonic acid, thiophosgene and *o*-nitrobenzaldehyde ;

(*b*) there is no effect on the magnetic susceptibility in the case of maleic acid, hydrazones and anils.

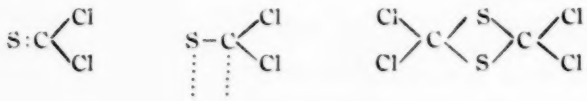
Cinnamylidene-malonic Acid.—Rüber (*loc. cit.*) showed that the yellow cinnamylidene-malonic acid on exposure to light polymerises and is transformed to a colourless acid which is a dimeride diphenyltetramethyldicarboxylic acid.



... (*i*)

Now as a result of the polymerisation of cinnamylidene-malonic acid, the mass susceptibility of the acid should rise as it polymerises, as polymerisation results in the disappearance of two double bonds. Instead of a rise however, there is a fall. This seems to be probably due to the formation of a ring on polymerisation according to equation (*i*). An analogous behaviour has been reported by Farquharson (*Trans. Faraday Soc.*, 1936, **32**, 219) in the case of the polymerisation of *cyclo*pentadiene, where too the disappearance of the double bonds and the formation of the ring results in a fall rather than in a rise of mass susceptibility.

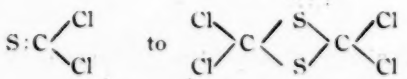
Thiophosgene.—Schonberg and Stephenson (*Ber.*, 1933, **66**, 567) showed that thiophosgene on exposure to light forms a white solid photodimer. Schonberg (*Trans. Faraday Soc.*, 1936, **32**, 574), suggested the appearance of diradical (II) during the process of photodimerisation and assigned constitution (III) to the photodimer.



(I) (II) (III)

If a diradical be formed as suggested by Schonberg, the liquid after exposure to light should show paramagnetism. Even if in the photoequilibrium the concentration of the diradical be small, it should be capable of being detected magnetically, because of the strong paramagnetism that a biradical should exhibit. But actualiy the value of the exposed liquid was found to be $-4{\cdot}394\times10^{-7}$ which is practically identical with the value for unexposed thiophosgene ($-4{\cdot}401\times10^{-7}$). From this the probable conclusion is that the suggested diradical is not formed or if it is formed its life is so short that it is incapable of being detected magnetically.

From the magnetic data given in Table I we find that on exposure to light, the magnetic susceptibility value of thiophosgene falls from $-4{\cdot}401\times10^{-7}$ to $-3{\cdot}914\times10^{-7}$. A change in the magnetic susceptibility does take place when a liquid solidifies but as a general rule the magnetic susceptibility rises when this happens. The only other explanation of this change is polymerisation. The polymerisation of thiophosgene on exposure to light is



accompanied by the disappearance of two double bonds between C:S and by the formation of a ring. A fall of the magnetic susceptibility observed may be due to the disappearance of double bonds, but this would result in a rise rather than a fall in the value. The fall may be attributed, therefore, to the formation of a closed ring analogous to that of cinnamylidene-malonic acid and *cyclo*pentadiene.

o-*Nitrobenzaldehyde.*—The effect of sunlight on *o*-nitrobenzaldehyde was first studied by Ciamician and Silber (*Ber.*, 1901, **34**, 2040). Since then this phenomenon has been investigated extensively both in the solid state as well as in solution and it has been shown that on exposure to light this substance is transformed into *o*-nitrosobenzoic acid. Lobry de Bruyn (*Rec. trav. Chim.*, 1903, **22**, 298) and later on Bowen and his collaborators (*J. Chem. Soc.*, 1924, **125**, 1218) while studying this phenomenon suggested that during the process of transformation of aldehyde to the acid, the green compound that is formed is the monomolecular variety, whereas the white acid that is formed ultimately is the bimolecular compound.

The magnetic susceptibility value of *o*-nitrobenzaldehyde, however, is $-0{\cdot}439\times10^{-6}$ and that of *o*-nitrosobenzoic acid $-0{\cdot}3926\times10^{-6}$. If the magnetic data tabulated in Table II be examined, it would be observed that the magnetic susceptibility of *o*-nitrobenzaldehyde in the solid state after an exposure of 12 hours is slightly higher ($-0{\cdot}443$) than that of the original compound, but on longer exposures it begins to decrease and ultimately after 8 days the value becomes constant at $-0{\cdot}393\times10^{-6}$, which is the magnetic susceptibility value for *o*-nitrosobenzoic acid. In solution also the value of the green compound is somewhat higher than that of *o*-nitrobenzaldehyde after a mild exposure to light. But when white *o*-nitrosobenzoic acid starts to separate out, the magnetic value falls. This suggests, therefore, that the magnetic susceptibility value of the green *o*-nitrosobenzoic acid is higher than that of the white variety. It has been shown that the polymerisation of a compound is accompanied with a change in magnetic susceptibility. The lower magnetic susceptibility value of the white *o*-nitrosobenzoic acid shows therefore that it is a polymer of the green variety as suggested by Bowen and his collaborators. In order to confirm this, the molecular weights of the two varieties were experimentally determined. The white *o*-nitrosobenzoic acid is insoluble in most liquid solvents. Therefore its molecular weight was determined by Rast's method, using camphor as the solvent and was found to be 292 whereas that of the green solution in benzene, by cryoscopic method, was found to be 151. Both from the magnetic and cryoscopic determinations, it can be inferred, therefore, that the green variety of *o*-nitrosobenzoic acid is monomolecular, whereas the white one is the polymerised variety.

Maleic Acid.—Berthelot and Gaudechon ("Travaux Scientifique de D." Berthelot, Paris, 1917) observed that maleic acid on exposure to light is transformed to fumaric acid. Kailan (*Z. physikal. Chem.*, 1914, **87**, 333) while studying this phenomenon, observed that the photochemical state in

the transformation of maleic to fumaric acid contains 75% maleic acid and 25% fumaric acid. If that be so, the theoretical magnetic susceptibility value of the exposed maleic acid would be $-0{\cdot}427 \times 10^{-6}$. The difference between the exposed and unexposed maleic acid is so little that it cannot be detected on the magnetic balance (Bhatnagar and Mathur, *Z. Physik*, 1930, **69**, 373).

Anils and Hydrazones.—Anils and hydrazones do not show any change in the magnetic susceptibility value on exposure to light. β-Naphthylamine, salicylidene, a lemon yellow compound, on exposure to light becomes red. The red variety is also obtained by crystallising the compound from alcoholic solution. Senier and Shepherd (*loc. cit.*) have shown that the red and the yellow bases, prepared under special conditions, are identical with those obtained by phototropy and they are related to each other as phototrops. From the magnetic data (Table I), it is clear that the magnetic susceptibility value of all the varieties is the same, showing that all of them are perhaps identical in constitution and that the difference in the colours may be due to their states of aggregation being different.

Disalicylidene-*m*-phenylenediamine is dimorphic. Senier, Shepherd and Clark showed that the two forms, plates and needles, are themselves phototropic and unlike β-naphthylamine-salicylidene, the two varieties are not related to each other as phototrops.

The magnetic susceptibilities of the two varieties, plates and needles, are $-4{\cdot}78 \times 10^{-7}$ and $-4{\cdot}19 \times 10^{-7}$ respectively. These values are not affected on exposure to light. The difference in magnetic susceptibility of the two varieties is due to the difference in crystal structure. If there had been a transformation of one variety to another on exposure to light, the magnetic susceptibility would have been changed. But as no change is observed on exposure to light, it shows that the two varieties are not related to each other as phototrops, but are themselves individually phototropic. This view is supported by the conclusions arrived at by Senier and Shepherd (*loc. cit.*) from their investigations.

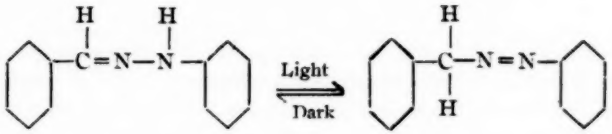
Padoa (*Atti. R. Accad. Lincei*, 1913, **22**, 1500) believes that the phototropic change involves a polymerisation and depolymerisation and that the weakly coloured dark stable forms are the drivers of the more strongly coloured light stable forms

$$A_2 \underset{\text{Dark}}{\overset{\text{Light}}{\rightleftharpoons}} 2A$$

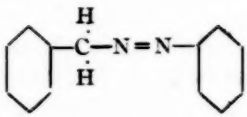
Dark stable — Light stable

This view is obviously not supported by the present investigation of the phototropic anils and hydrazones, for if polymerisation had taken place it would have been accompanied by a change in the magnetic susceptibility of the compound on exposure to light as for example in the case of anthracene, thiophosgene, cinnamylidene-malonic acid which have been shown to polymerise under the influence of light.

Chattaway (*J. Chem. Soc.*, 1906, **89**, 462) believed that in case of hydrazones, *e.g.*, benzaldehyde-phenylhydrazone, a chemical action takes place and suggested the equilibrium

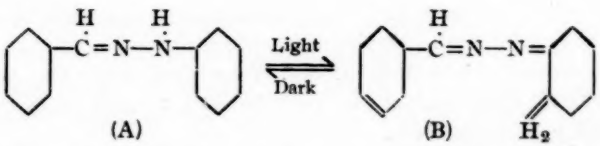


On the other hand, Gallagher (*Bull. Soc. chim.*, 1921, *iv*, **29**, 683) synthesised the substance



and found that it was stable both in dark and in light. From this he concluded that phototropy of hydrazones could not be due to the isomeric change as suggested by Chattaway (*loc. cit.*).

As a result of extensive investigations on the phototropy of hydrazones, Craziani and Borini (*Atti. R. Accad. Lincei*, 1913, **22**, 32) suggested the mechanism:



in which they postulated the formation of a hypothetical compound B. On Pascal's additivity law, the magnetic susceptibility of benzaldehyde-phenylhydrazone should be $-0{\cdot}607\times10^{-6}$ and that of compound B should be $-0{\cdot}592\times10^{-6}$. But actually the magnetic values both for the exposed and the unexposed samples are identical (*vide* Table I). From this it may be concluded that the hypothetical compound is not formed.

This conclusion is, however, open to the criticism that the above noted constancy in the value of the magnetic susceptibility on exposure to light may be due to the formation of a mere trace of the compound B under the circumstances. An experiment was, therefore, devised to cause a considerable amount of change by powdering large crystals to colloidal dimensions, at the same time exposing them to bright light. This resulted in considerable deepening of the colour due to the formation of a sufficient quantity of the supposed photo-isomer, but no change in the magnetic susceptibility value of the powder was noticeable. From this the most probable conclusion that can be drawn is that the compound B is not formed on exposure to light.

The only view which can be supported from the magnetic data is the one put forward by Senier and Shepherd according to which the phototropy of anils and hydrazones is attributed to the change in the aggregates under the action of light, different aggregates having different colours. That a mere change in the aggregate sizes does not cause a change in the value of susceptibility having been fully established by Bhatnagar and his collaborators (*J. Indian Chem. Soc.*, 1933, **10**, 321 ; *Kolloid Z.*, 1937, **78**, 9 ; Lessheim, *Current Sci.*, 1936, **5**, 119). It may be suggested, however, that if due to change in aggregate sizes long chain anisotropic molecules are formed, the change in susceptibility may be possible.

A view which has been recently propounded is that the phototropy of anils and hydrazones may be due to the distortion of electronic orbits within a crystal (Lyman Chalkley Jr., *Chem. Rev.*, 1929, **5**, 217). The only support for this view at present is that the change in colour in the case of β-naphthylamine-salicylidene can also be brought about by agencies other than light. From the present data no definite conclusion for and against this view can be drawn, although the fact that increasing quantities of coloured materials are formed on colloidalisation of substances only in presence of light, seems to be against the theory of distortion of electronic orbits within the crystal. Further work is in progress.

The authors wish to thank Messrs. Varma and Dhir who were associated with the earlier part of the work but had to give up on account of their employments elsewhere.

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DENITRIFICATION IN SUNLIGHT AND ITS RETARDATION PART IV.

By N. R. Dhar and S. K. Mukerji.

Experiments show that when ammonium sulphate in solution is mixed with substances like titanium oxide, zinc oxide, silica, sterile or unsterile soil, there is appreciable nitrification and loss of nitrogen even in the dark, under completely sterile conditions. The nitrification and nitrogen lost increase in light. Before the formation of nitrate there is always the production and decomposition of ammonium nitrite in the process of nitrification. In all cases the actual amount of nitrate present is small in comparison with the loss of nitrogen. Hence, the amount of nitrate actually present in a soil after the addition of nitrogenous manure is not a true measure of nitrification which has to be determined by the difference in the total nitrogen before and after the process rather than on the actual amount of nitrate present.

Very little ammonia is lost from the soil as free ammonia although considerable amount of nitrogen loss takes place from soils.

Very little loss of nitrogen is observed when a nitrate is mixed with glucose and exposed to sun light.

Under field conditions on the addition of blood, urea and gelatin there is more loss in light than in dark. Molasses readily conserve the total nitrogen in the soil, and that is why organic substances are useful in preserving and increasing the soil nitrogen.

In recent years (*J. Indian Chem. Soc.*, 1935, **12**, 77, 756 ; 1936, **13**, 555) we have shown that when ammonium salts or other nitrogenous compounds undergo nitrification in the soil or in solutions there is a considerable loss of nitrogen in the gaseous state due to the formation and decomposition of ammonium nitrate, specially when the dose of nitrogen added is high. This loss is intimately connected with the process of nitrification and appears to be unavoidable in the soil under normal conditions. Field trials with different manures at Rothamsted and at other places reveal as high a loss as 70% of the added nitrogenous manure.

We have carried on further experiments on this type of nitrogen loss under aerobic conditions with ammonium salts in contact with soil and surfaces like TiO_2, ZnO, SiO_2 etc. under completely sterile conditions. The dark experiments were carried on in flasks completely covered with black cloth. The following are the experimental results. The garden soil used in some of these experiments contained NH_3-N = 0·7 mg.%, NO_3-N = 1.1 mg.% and Total N = 33 mg.%.

The p_H of the original soil was 7·6. Most of the experiments described in this paper have been repeated several times.

TABLE I.

Started on 14-9-37 and stopped on 27-12-37. Exposure to sunlight for 604 hours. Max. temp. = 40°. Min. temp. = 23° in 500 c.c. conical Jena flask plugged with cotton wool.

Light.

Added	Left				
	NH_3-N. %	NO_3-N. %	Total N. %	%Loss.	p_H.
5 g. TiO_2 + 0·0175 g. NH_3-N + 100 c.c. water	0·0112 g.	0·002 g.	0·0118 g.	21·1	6·7
5 g. SiO_2 + „ „ + „	0·0056	Nil	0·0058	66·8	6·6
5 g. ZnO + „ „ + „	0·0003	0·0035	0·004	77	7·2
5 g. Sterile soil + „ „ + „	0·0012	Nil	0·0074	57	7·4
5 g. Unsterile soil + „ „ + „	0·0008	„	0·0056	67	7·4

Dark.

5 g. TiO_2 + 0·0175 g. NH_3-N + 100 c.c. water	0·014 g.	„	0·0144 g.	19	6·9
5 g. SiO_2 + „ „ + „	0·014	„	0·0144	19	6·8
5 g. ZnO + „ „ + „	0·010	„	0·0124	28	7·2
5 g. Sterile soil + „ „ + „	0·007	„	0·0098	50·8	7·4
5 g. Unsterile soil + „ „ + „	0·0014	0·0093	0·0067	8·5	6·8

Started on 14-9-37 and stopped on 28-12-37. Exposure to sunlight for 618 hours. Max. temp. = 40°. Min. temp. = 23° in 500 c.c. conical Jena flask plugged with cotton wool.

Light.

5 g. TiO_2 + 0·035 g. NH_3-N + 100 c.c. water	0·0282 g.	0·0046 g.	0·028 g.	7	6·7
5 g. SiO_2 + „ „ + „	0·0112	Nil	0·0121	65	6·2
5 g. ZnO + „ „ + „	0·0093	0·0038	0·0098	61	7·6
5 g. Sterile soil + „ „ + „	0·0056	Nil	0·0172	50	6·7

Dark.

5 g. TiO_2 + 0·035 g. NH_3-N + 100 c.c. water	0·0318 g.	Nil	0·0321 g.	8·3	6·8
5 g. SiO_2 + „ „ + „ „	0·0188	„	0·0298	14 8	6·0
5 g ZnO + „ „ + „ „	0·0112	„	0·0148	57·7	7·6
5 g. Sterile soil + „ „ + „ „	0·014	„	0·028	20·0	6·6

TABLE I (*contd.*).

Started on 14-9-37 and stopped on 3-1-38. Exposure to sunlight for 646 hours. Max. temp. = 40°. Min. temp. = 23° in 500 c.c. conical Jena flask plugged with cotton wool.

Light.

Added				Left				
				NH_3-N. %	NO_3-N. %	Total N. %	% Loss.	p_H.
5 g. TiO_2	+ 0·07g.	NH_3–N	+ 100 c.c. water	0·0467 g.	0·0062 g.	0·0534 g.	18·8	6·0
5 g. SiO_2	+ ,,	,,	+ ,, ,,	0·035	Nil	0·0362	48·2	6·0
5 g. ZnO	+ ,,	,,	+ ,, ,,	0·014	0·0046	0·0192	64·5	—
5 g. Sterile soil	+ ,,	,,	+ ,, ,,	0·0254	Nil	0·0314	55·1	6·4
5 g. Unsterile soil	+ ,,	,,	+ ,, ,,	0·0164	Nil	0·0366	47·6	6·2

Dark.

5 g. TiO_2	+ 0·07 g.	NH_3-N	+ 100 c.c. water	0·0638 g.	Nil	0·0642	8·3	6·0
5 g. SiO_2	+ ,,	,,	+ ,, ,,	0·0508	,,	0·0512	26·8	6·0
5 g. ZnO	+ ,,	,,	+ ,, ,,	0·0221	,,	0·0292	57·4	—
5 g. Sterile soil	+ ,,	,,	+ ,, ,,	0·0468	,,	0·0564	19·4	6·2
5 g. Unsterile soil	+ ,,	,,	+ ,, ,,	0·0508	0·0014	0·0588	16·0	6·2

Started on 14-9-37 and stopped on 6-1-38. Exposure to sunlight for 667 hours. Max. temp. = 40°. Min. temp. = 23° in 500 c.c. conical Jena flask plugged with cotton wool.

Light.

				NH_3-N. %	NO_3-N. %	Total N. %	% Loss.	p_H.
5 g. TiO_2 +	0·14 g.	NH_3-N	+ 100 c.c. water	0·11 g.	0·007 g.	0·112 g.	15·0	5·6
5 g. SiO_2 +	,,	,,	+ ,, ,,	0·084	0·0009	0·0862	37·8	5·8
5 g. ZnO +	,,	,,	+ ,, ,,	0·030	0·0049	0·0312	74·0	7·2
5 g. Sterile soil +	,,	,,	+ ,, ,,	0·062	0·0007	0·0968	30·8	5·6
5 g. Unsterile soil +	,,	,,	+ ,, ,	0·051	0·0007	0·1041	25·4	5·8

TABLE I (*contd.*).

Dark.

Added	Left				
	NH_3-N. %	NO_3-N. %	Total N. %	% Loss.	p_H.
5 g TiO_2+ 0·14 g. NH_3-N+100 c.c. water	0·128 g.	Nil	0·131	6·5	5·6
5 g. SiO_2 + „ „ + „ „	0·112	„	0·119	15·0	5·8
5 g. ZnO + „ „ + „ „	0·0904	„	0·0928	33·8	7·0
5 g. Sterile soil + „ „ + „ „	0·123	„	0·131	6·5	5·8
5 g. Unsterile soil + „ „ + „ „	0·117	0·0005	0·131	6·5	5·8

Started on 14-9-37 and stopped on 10-1-38. Exposure to sunlight for 675 hours. Max. temp. = 40°. Min. temp = 23° in quartz boiling tube. Sterile and plugged with cotton wool

Light.

5 g. TiO_2+ 0·14 g. NH_3 N+100 c.c. water	0·0934 g.	0·0031 g.	0·0944 g.	30·3	5·6
5 g. SiO_2 + „ „ + „ „	0·052	0·0011	0·052	62·0	5·8
5 g. ZnO + „ „ + „ „	0·0467	0·0011	0·0472	66·4	7·0
5 g. Sterile soil + „ „ + „ „	0·0519	0·0008	0·056	59·4	6·2

Started on 28-1-38 and stopped on 17-5-38. Exposure– daily 8 hours in 1000 watt bulb light in 500 c c. Jena flask plugged with cotton wool.

5 g. TiO_2+0·14 g NH_3-N+100 c.c. water	0·1286 g.	0·0184 g.	0·1214 g.	0·14	5·6
5 g. ZnO_2+ „ „ + „ „	0·0896	0·0386	0·0984	2·15	7·0
5 g. TiO_2+0·07 g. „ + „ „	0·0584	0·0110	0·0512	1·11	6·0
5 g. ZnO+0·07 g. „ + „ „	0·0229	0·0268	0·0321	15·7	7·2

The experiments recorded above show that even in the dark there is appreciable nitrification and loss of nitrogen when ammonium salts in solutions are mixed with surfaces and kept under sterile conditions. The nitrification and nitrogen loss are always greater in light than in the dark. Moreover, the large number of experimental results recorded in this paper show conclusively that nitrification and nitrogen loss are possible

even in the absence of bacteria both in light and in the dark. It appears, therefore, that ammonium salts can undergo slow oxidation on different surfaces with the formation of nitrites and nitrates even in the absence of nitrite or nitrate formers (bacteria). The oxidation, however, is greater if the mixture of the ammonium salts and surface contains bacteria or is exposed to light. It must be emphasised that under normal conditions in the soil or in solutions there is more loss of nitrogen due to the formation and decomposition of ammonium nitrite than the formation of nitrate. It is well known that under normal conditions, soil contains small quantities of nitrate and traces of nitrite, although the nitrogen content may be fairly high.

The marked influence of zinc oxide in helping nitrogen loss in light cannot be ascribed to the reaction of ammonium salts with zinc oxide, because this action should be more or less identical in both light and in the dark, as the temperature of the vessels in light and dark was almost the same.

When the nitrogenous matter undergoes oxidation on the soil surface, the first product in the case of proteins is ammonia, which on nitrification produces nitrites and nitrates. In the case of ammonium salts, by nitrification, nitrites and nitrates are produced. It appears, therefore, that before the formation of nitrate, there is always the possibility of the loss of nitrite by the formation and decomposition of ammonium nitrite as an intermediate product. Hence in all cases the actual formation of nitrate may be small in comparison with the loss of nitrogen by the process of the decomposition of ammonium nitrite. Our experimental results are in agreement with these views, because in most cases the amount of nitrate present after the process of nitrification is small in comparison with the nitrogen lost. It is clear, therefore, that the amount of nitrate actually present in a soil or a solution on the addition of nitrogenous manure is not a true measure of nitrification, which causes more loss of nitrogen in the gaseous state than the amount of the nitrate formation. Hence the amount of nitrification under aerobic conditions has to be judged by the difference in the total nitrogen before and after the process, rather than on the actual quantity of nitrate present. Hence in order to estimate the amount of nitrification under ordinary conditions in a system it is necessary to estimate the total nitrogen before and after the process. The recent experiments of Waksman and Madhok (*Soil Sci.*, 1937, **44**, 361) are not at all convincing as the total nitrogen of the soil before and after the experiments has not been determined. Unless the difference of total nitrogen before and after the experiments is known, it is impossible to conclude definitely about the amount of nitrification.

We have repeatedly observed that hardly any ammonia escapes under aerobic conditions in the soil, because in our experiments no ammonia was obtained in flasks containing sulphuric acid, although large amounts of nitrogen gas was collected in the nitrometer.

Amount of gas collected when water was put into the nitrometer. Temperature = 30°. Time = 37 days.

200 G. soil + 0·28 g. nitrogen + 50 c.c. water = 99·5 c.c. gas

,, + 50 c.c. water only = 21 c.c. gas

Amount of gas collected by the displacement of 20% caustic potash in the nitrometer. Temperature = 32-33°. Time = 52 days.

200 G. soil + 0·28 g. nitrogen + 50 c.c. water = 11·3 c.c.

,, + 50 c.c. water = 1·8 c.c.

It may be stated that the nitrogen may be lost as ammonia gas from the soil, but this seems improbable in the soils of temperate climates, because they are acidic in nature. In order to test this point with our soils the following experiments have been performed.

Three 250 c.c. conical flasks containing concentrated sulphuric acid, ferrous sulphate and sulphuric acid and distilled water respectively were joined in series to a quartz flask of 250 c.c. capacity. To the other end of the quartz flask, three flasks containing dilute sulphuric acid were attached to absorb the ammonia evolved from the reaction vessel. A mixture of sterile soil and ammonium sulphate was put in the reaction vessel and was sterilised and plugged with cotton wool. Air from a gas cylinder of 30 litres capacity was bubbled through these flasks. After the exposure, the contents of the quartz flask as well as of the catches were analysed. The following are the experimental results.

TABLE II.

Started on 13-1-37 and stopped on 8-2-37. Exposure—150 hours to sunlight in quartz flask (250 c c.). Aeration—30 litres for each 7 hours of exposure.

Initial concentration.	Final concentration.
NH_3-N = 0·0483%	0·02084%
NO_3-N = 0·0022	0·0025
Total N = 0·0877	0·0759
	3·7% lost as calculated from ammonia
	Ammonia in the catches placed next to the reaction vessel = 0·0033 g.
	Loss in total nitrogen = 0·0127 g. or 14·4%

The experimental results recorded above show that the loss of nitrogen cannot be accounted for on the viewpoint, that gaseous ammonia is chiefly lost because the loss of total nitrogen is much greater than that of ammonia.

Once the nitrate is formed, it can be lost by leaching and not by denitrification of the type discussed in this paper as is evident from the following results.

TABLE III.

Started on 8-9-36 and stopped on 6-10-36. Exposure to sunlight for 100 hours.

Added	Left		
	NH_3-N.	NO_3-N.	Total N.
1 g. Fe_2O_3 + 1·4 g. NO_3-N + 100 c.c. water	0·002 g.	1·381 g.	1·392 g.
,, + 0·7 ,, + ,,	0·002	0·648	0·6684
,, + 0·14 ,, + ,,	0·002	0·1236	0·1366
,, + 0·07 ,, + ,,	0·0019	0·0618	0·0654
,, + 0·035 ,, + ,,	0·0010	0·0124	0·0139
,, + 0·0028 ,, + ,,	0·0007	0·0020	0·0028
,, + 0·014 ,, + ,,	0·001	0·0124	0·0139

Started on 27-10-36 and stopped on 9-12-36. Exposure to sunlight for 200 hours.

1 g. Fe_2O_3 + 1·4 g. NO_3-N + 100 c.c. water	0·0007 g.	1·388 g.	1·4 g.
,, + 0·7 ,, + ,,	0·0003	0·6982	0·7
,, + 0·14 ,, + ,,	0·0001	0·139	0·1389
,, + 0·07 ,, + ,,	0·0012	0·0688	0·07
,, + 0·035 ,, + ,,	0·0016	0·0298	0·0348
,, + 0·014 ,, + ,,	0·0015	0·0132	0·014
,, + 0·0028 ,, + ,,	0·0004	0·0020	0·0028

Started on 8-9-36. Stopped on 6-10-36. Exposure to sunlight for 100 hours.

1 g. Fe_2O_3 + 1 g. glucose + 1·4 g. NO_3-N + 100 c.c. water	0·0056 g.	0·948 g.	1·3 g.
,, + ,, ,, + 0·7 + ,,	0·0056	0·586	0·644
,, + ,, ,, + 0·14 + ,,	0·0046	0·112	0·132
,, + ,, ,, + 0·07 + ,,	0·004	0·0467	0·066
,, + ,, ,, + 0·035 + ,,	0·0028	0·0200	0·02722
,, + ,, ,, + 0·014 + ,,	0·0012	0·00406	0·01316
,, + ,, ,, + 0·0028 + ,,	0·0008	0·0004	0·0031

TABLE III (*contd.*).

Started on 27-10-36. Stopped on 9-12-36. Exposure to sunlight for 200 hours.

					NH_3-N.	NO_3-N.	Total N.
1 g.	Fe_2O_3 + 1 g.	glucose	+ 1·4 g.	NO_3-N + 100 c.c. water	0·007 g.	0·9324 g.	1·4 g.
,,	+ ,,	,,	+ 0·7	+ ,,	0·0062	0·5724	0·7
,,	+ ,,	,,	+ 0·14	+ ,,	0·0058	0·0998	0·1398
,,	+ ,,	,,	+ 0·07	+ ,,	0·0052	0·0456	0·07
,,	+ ,,	,,	+ 0·035	+ ,,	0·0031	0·0192	0·0348
,,	+ ,,	,,	+ 0·014	+ ,,	0·0021	0·0038	0·014
,,	+ ,,	,,	+ 0·0028	+ ,,	0·0009	0·0001	0·0039

It is generally believed that denitrification is frequently observed when a nitrate is mixed with organic matter but in our experiments the beakers containing nitrates alone do not show appreciable nitrogen loss or a change of nitric nitrogen. The beakers containing glucose and nitrate in some cases show a decrease in nitrate, but not in the total nitrogen, and at the lowest concentration when the carbon-nitrogen ratio is above 20, nitrogen fixation takes place.

Blood, urea, and gelatin were added to small plots in fields. The ammoniacal, nitric, total nitrogen and total carbon were determined from time to time. Some of the fields have been treated with molasses and some are covered with wooden planks to determine the effect of sunlight and carbonaceous substances upon nitrification and nitrogen loss from soils. The following results show that our conclusions regarding the phenomenon of nitrogen loss are also being borne out by field experiments. The first estimation shows the condition of the field just after mixing the above substances.

TABLE IV.

Blood.

Plot 6 sq. ft. Started on 27-11-36.

Light.

NH_3-N. %	NO_3-N. %	Total N. %	Total C. %	Date of sampling.
0·001 g.	0·0028 g.	0·07 g.	0·5424 g.	27-11-36
0·0012	0·0031	0·07	0·5133	7-12-36
0·0025	0·0037	0·07	0·4569	25-12-36
0·0026	0·0042	0·07	0·4562	25-1-37
0·0009	0·008	0·0636	0·4421	25-2-37
0·0011	0·0112	0·05	0·4412	22-3-37
0·0011	0·0116	0·0482	0·4421	3-5-37
		Covered.		
0·0010 g.	0·0032	0·0823	0·5569	27-11-36
0·0012	0·0034	0·0823	0·5234	7-12-36
0·0022	0·0036	0·0799	0·4932	25-12-36
0·0021	0·0038	0·0799	0·4884	25-1-37
0·0014	0·0073	0·0636	0·4646	25-2-37
0·0014	0·008	0·0584	0·4622	22-3-37
0·0014	0·0082	0·0578	0·4612	3-5-37

TABLE V.

Blood + molasses.

Plot 6 sq. ft. Started on 30-11-36.

Light

NH_3-N. %	NO_3-N. %	Total N. %	Total C. %	Date of sampling.
0·0031 g.	0·0032 g.	0·0934 g.	2·829 g.	30-11-36
0·0029	0·0031	0·0931	2·539	7-12-36
0·0037	0·0032	0·0934	2·182	25-12-36
0·0043	0·0035	0·0941	1·1214	25-1-37
0·0031	0·0021	0·0931	0·8624	25-2-37
0·0028	0·0035	0·0778	0·8452	22-3-37
0·0028	0·0036	0·0712	0·8314	3-5-37

Covered

0·0028	0·0022	0·0875	2·781	30-11-36
0·0020	0·0025	0·0875	2·631	7-12-36
0·0056	0·0022	0·0874	2·010	25-12-36
0·0056	0·0018	0·0872	0·9921	25-1-37
0·0018	0·0014	0·0824	0·7212	25-2-37
0·0029	0·0031	0·0824	0·7224	22-3-37
0·0029	0·0031	0·0823	0·7112	3-5-37

TABLE VI.

Urea.

Plot 6 sq. ft. Started on 7-12-36.

Light

0·0031 g.	0·0022 g.	0·14 g.	0·385 g.	7-12-36
0·0209	0·0031	0·14	0·388	25-12-36
0·02	0·0050	0·1272	0·3892	25-1-37
0·015	0.0086	0·1178	0·3892	25-2-37
0·0035	0·028	0·0875	0·3921	22-3-37
0·0036	0·0318	0·0710	0·3921	3-5-37

Covered

0·0040	0·0023	0·175	0·385	7-12-36
0·0146	0·0025	0·175	0·3886	25-12-36
0·0155	0·0039	0·1654	0·3892	25-1-37
0·0017	0·0070	0·1524	0·3892	25-2-37
0·0014	0·0250	0·1004	0·3892	22-3-37
0·0016	0·027	0·0998	0·3892	3-5-37

TABLE VII.

Urea + molasses.

Plot 6 sq. ft. Started on 7-12-36.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
0·0050 g.%	0·0023%	0·200%	2·553%	7-12-36
0·0056	0·0020	0·1986	2·079	25-12-36
0·0062	0·0043	0·1925	0·9994	25-1-37
0·0031	0·0044	0·1612	0·8132	25-2-37
0·0031	0·0056	0·1277	0·8043	22-3-37
0·0037	0·0062	0·1182	0·8112	3-5-37
		Covered.		
0·0049	0·0034	0·220	3·431	7-12-36
0·0043	0·0028	0·1892	1·5831	25-12-36
0·0061	0·0037	0·1874	0·8454	25-1-37
0·0043	0·0039	0·1721	0·7944	25-2-37
0·0037	0·0040	0·1346	0·7934	22-3-37
0·0037	0·0054	0·1212	0·7118	3-5-37

TABLE VIII.

Gelatin.

Plot 6 sq. ft. Started on 25-12-36.

		Light.		
0·02 g%	0·0056%	0·200%	0·7198	25-12-36
0·04	0·0062	0·200	0·7112	25-1-37
0·046	0·0074	0·1752	0·7086	25-2-37
0·035	0·007	0·1556	0·6012	22-3-37
0·014	0·0102	0·1042	0·6102	3-5-37
		Covered.		
0·02 g%	0·0056	0·212	0·7198	25-12-36
0·037	0·0058	0·200	0·7154	25-1-37
0·038	0·0063	0·1823	0·7092	25-2-37
0·0174	0·0075	0·1667	0·6124	25-3-37
0·0178	0·0079	0·1508	0·6124	3-5-37

TABLE IX.

Gelatin + molasses.

Plot 6 sq. ft. Started on 25-12-36.

Light.

NH_3-N.	NO_3-N.	Total N.	Total C.	Date of sampling.
	%	%	%	
0·020 g.%	0·0062 g.	0·200	1·838	25-12-36
0·0056	0·0066	0·200	0·9842	25-1-37
0·0181	0·0047	0·1924	0·8218	25-2-37
0·0056	0·008	0·175	0·8138	22-3-37
0·0062	0·0084	0·1731	0·8034	3-5-37

Covered.

0·020	0·008	0·200	1·838	25-12-36
0·007	0·0057	0·200	0·9672	25-1-27
0·0106	0·0039	0·1864	0·8122	25-2-37
0·005	0·028	0·175	0·8122	22-3-37
0·0056	0·030	0·1734	0·8122	3-5-37

The experiments carried on in flasks show that although the dark vessels had slightly a higher temperature than the vessels kept in sunlight, the loss in total nitrogen due to nitrification is always greater in vessels receiving sunshine than in those kept in the dark when ammonium sulphate at various concentrations were mixed with surface like titanium oxide, silica, zinc oxide and sterile or unsterile garden soil. It appears, therefore, that sunlight, which increases the velocity of nitrification, also increases nitrogen loss.

The field trials carried on with different nitrogenous substances like blood, urea and gelatin show that even in the field trials there is more marked nitrogen loss from the fields exposed to sunlight than in those covered with wooden planks. Another highly significant result has been obtained in these experiments, that when molasses is mixed with these nitrogenous substances and added to the field, the nitrogen loss in the fields

both in light and in the dark is much smaller than in those plots where nitrogenous substances were added to the soil alone. It is clear, therefore, that the carbonaceous substances present in molasses retard the process of nitrification and the loss of nitrogen. Hence in tropical soils, at any rate, a mixture of nitrogenous substances with carbonaceous substances like molasses, hay, leaves, etc, are more useful as a nitrogenous store than the nitrogenous substance alone.

For the first time in the study of the problem of organic manures, light has been thrown by our researches on the value of organic nitrogenous manure. The carbonaceous substances added with the organic manure not only protect nitrogen and retard loss, but also in many cases when the proportion of carbonaceous subtances is large, fixation of nitrogen takes place as in the case of cowdung and other substances.

It has been frequently mentioned that the loss of nitrogen observed after the addition of nitrogenous substances to the soil is due to the escape of ammonia, but from the experiments described in these papers, it appears that the amount of ammonia that escapes from the soil mixed with ammonium sulphate is very small although a good deal of nitrogen is lost from soil due to the formation and decomposition of ammonium nitrite found as an intermediate product during nitrification. Hence the nitrogen loss under aerobic condition cannot be explained from the viewpoint of escape of ammonia.

It is of interest to note that in our field trials in light with the nitrogeneous substances mixed with molasses, sometimes in the beginning of of the experiments, the loss in nitrogen may be slightly less than that in the dark. This is noticeable with blood and gelatine mixed with molasses. We are of opinion that this behaviour may be due to a certain amount of nitrogen fixation in light in the molassed fields containing nitrogneous compounds.

We have also investigated the influence of temperature on nitrification of ammonium sulphate mixed with soil. The following results have been obtained.

TABLE X.

200 G. soil + 0·28 g. of N as ammonium sulphate. Original NH_3-N = 0·00084 g.%. NO_3-N = 0·0036 g.% and total N = 0·0387 g.%.

At 20°.

NH_3-N.	NO_3-N.	Total N.	Date of sampling original soil.
%	%	%	
0·1468 g.	0·0036 g.	0·1787 g.	15-9-37

TABLE X (*contd*).

NH_3-N. %	NO_3-N. %	Total N. %	Date of sampling original soil.
0·08 g.	0·0048 g.	0·1167 g.	30-9-36
0·04	0·0064	0·1012	15-10-87
0·022	0·0188	0·0964	30-10-37
	At 25°.		
			Original soil
0·1408	0·0036	0·1787	15-9·37
0·074	0·0052	0·1129	30-9·37
0·035	0·0162	0·0934	15-10·37
0·0198	0·0268	0·0912	30-10-37
	At 30°.		
			Original soil
0·1408	0·0036	0·1787	15-9·37
0·046	0·0080	0·1129	30-9-37
0·0062	0·0372	0·070	15-10-37
0·0042	0·0384	0·0668	30-10-37
	At 35°.		
			Original soil
0·1408	0·0036	0·1787	15-9-37
0·050	0·0072	0·1078	30-9-37
0·008	0·028	0·0636	15-10-37
0·0042	0·0302	0·0612	30-10-37
	At 40°.		
0·1408	0·0036	0·1787	15-9-37
0·027	0·0058	0·0875	30-9-37
0·0086	0·0224	0·0603	15-10-37
0·0042	0·0242	0·0575	30-10-37
	At 45°.		
			Original soil
0·1408	0·0036	0·1787	15-9-37

TABLE X (*contd*).

NH_3-N. %	NO_3-N. %	Total N. %	Date of sampling.
0·024 g.	0·0048 g.	0·0700 g.	30-9-37
0·0092	0·0063	0·0583	15-10-37
0·0042	0·0064	0·0512	30-10-39
	At 50°.		
0·1408	0·0036	0·1787	15-9-37
0·016	0·004	0·0626	30-9-37
0·0092	0·0042	0·0538	15-10-37
0·0036	0·0042	0·0487	30-10-37
	At 60°.		
0·1408	0·0036	0·1787	15-9-37
0·0092	0·0036	0·0612	30-9-39
0·0092	0·0037	0·0538	15-10-37
0·0032	0·0036	0·0488	30-10-37
	Exposed to sunlight daily for 8 hours (temperature 35°).		
0·1408	0·0036	0·1787	15-9-37
0·0186	0·0037	0·0612	30-9-37
0·0092	0·0042	0·056	15-10-37
0·0042	0·0042	0·0492	30-10-37

TABLE XI.

200 G. soil + 8 g. cane sugar + 0·28 g. nitrogen as ammonium sulphate. The soil contained NH_3-N = 0·0004 g.%, NO_3-N = 0·002 g.% and total N = 0·0368 g.%. Started on 3-11-37.

At 10-13°.

NH_3-N. %	NO_3-N. %	Total N. %	Total C. %	Date
0·1404 g.	0·002 g	0·1768 g.	2·004 g.	3-11-37
0·116	0·0021	0·1676	1·2136	18-11-37

TABLE XI (*contd.*).

NH_3-N.	NO_3-N_2	Total N.	Total C.	Date.
%	%	%	%	
0·0824 g.	0·0022 g.	0·1489 g.	0·7770 g.	3-2-37[1]
0·053	0·0031	0·1273	0·7112	18-12-37
		At 25°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·070	0·0036	0·1676	0·9877	18-11-37
0·0572	0·0050	0·1278	0·556	3-12-37
0·028	0·0062	0·1167	0·5022	18-12-37
		At 30°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·043	0·0046	0·14	0·8956	18-11-37
0·0224	0·008	0·1167	0·5524	3-12-37
0·02	0·0104	0·100	0·4986	18-12-37
		At 35°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·040	0·008	0·1165	0·5273	18-11-37
0·020	0·0196	0·1016	0·5376	3-12-37
0·0104	0·025	0·0934	0·4195	18-12-37
		At 40°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·031	0·0032	0·1128	0·4205	18-11-37
0·017	0·0042	0·1000	0·4205	3-12-37
0·0085	0·0071	0·0875	0·4205	18-12-37
		At 45°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·0155	0·0032	0·1000	0·4205	18-11-37
0·0106	0·0036	0·0934	0·4205	3-12-37
0·0074	0·0043	0·0814	0·4205	18-12-37

TABLE XI (*contd.*).

NH_3 N. %	NO_3 N. %	Total N. %	Total C.	Date of sampling.
		At 50°.		
0·1404 g.	0·002 g.	0·1768 g.	2·004 g.	3-11-37
0·0132	0·0028	0·1000	0·6914	18-11-37
0·0104	0·0018	0·0934	0·5199	3-12-37
0·0062	0·0030	0·0778	0·5022	18-12-37
		At 60°.		
0·1404	0·002	0·1768	2·004	3-11-37
0·0096	0·002	0·1000	0·5312	18-11-37
0·0093	0·002	0·0885	0·5244	3-12-37
0·0015	0·002	0·0778	0·5187	18-12-37
		Exposed to sunlight. (Temperature 35°)		
0·1404	0·002	0·1768	2·004	3-11-37
0·02	0·003	0·1183	0·5273	18-11-37
0·016	0·0033	0·100	0·5010	3-12-37
0·007	0·0034	0·100	0·4986	18-12-37

The foregoing results show that the higher the temperature, the greater the loss of nitrogen. In sunlight, however, the loss in nitrogen is much greater than in the dark at the same temperature.

Canesugar markedly retards the loss of nitrogen when it is added to ammonium sulphate which is undergoing nitrification.

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MECHANISM OF THE MICROBIOLOGICAL OXIDATION OF AMMONIA. PART I. FORMATION OF INTERMEDIATE PRODUCTS.

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The microbiological oxidation of amn onia to nitrite has been studied with particular reference to the formation of any possible intermediate products. Nitrite forming bacteria isolated from soils obtained from different parts of India have been used in the present investigation. Hydroxylamine and hyponitrite expected from theoretical considerations could not be detected in any of the numerous cultures examined by the known qualitative tests, some of which are very sensitive.

It has been known for a long time that ammonium salts, when added to the soil undergo oxidation to nitrite and nitrate. This transformation of ammonia to nitrate is commonly known as nitrification. It was Pasteur (*Compt. rend.*, 1862, **54**, 265), who first suggested that the oxidation of ammonia is accomplished by micro-organisms. This was actually proved to be the case by two French Chemists, Schlosing and Muntz (*compt. rend.*, 1877, **84**, 401; **85**, 1018). They found that if a solution of ammonia was allowed to percolate through soil, which was well aerated at regular intervals, the ammonia was oxidised to nitrate. If the soil was heated to 100° or treated with antiseptics like chloroform it lost its capacity for oxidising ammonia. Warington (*J. Chem. Soc.*, 1878, **33**, 44) further confirmed the biological nature of nitrification not only in soil but also in ammoniacal solutions seeded with soil. After many investigators have vainly endeavoured to isolate the organisms in pure culture, the Russian bacteriologist Winogradsky succeeded in obtaining a pure culture using silicic acid gel as the solid medium.

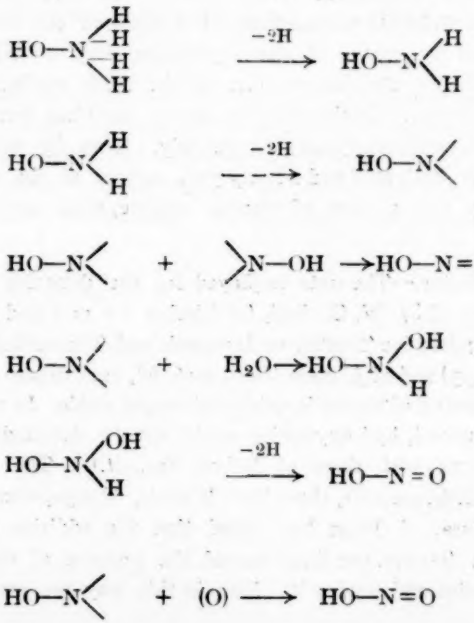
The researches of Winogradsky established that nitrification is really due to the joint action of two organisms : The nitrosomonas and nitrosococcus oxidise ammonia to nitrite; and nitrobacter oxidises nitrite to nitrate. The organisms are highly specific in character, the ammonia-oxidising bacteria have no action on nitrite, and the nitrite-oxidising organisms are without influence on ammonia. Since the pioneering work of Winogradsky much work has been done on the characteristics of these organisms by numerous investigators, notably Omeliansky (*Centl. Bakt.*, 1899, **2**, 537), Meyerhof (*Fflugers. Arch. Ges. Physiol.*, 1916, **164**, 353; **165**, 229; **166**, 240), Bonazzi (*J. Bact.*, 1923, **8**, 343), Boljes (*Archiv Mikrobiol.*, 1935, **6**, 79), Gopala Rao and Pandalai (*ibid.*, 1936, **7**, 32).

Very little is known about the chemical course of oxidation. Mumford (*Proc. Chem. Soc.*, 1914, 36; *cf.* Fowler, "Biochemistry of Nitrogen Conservation," 1934, p. 99) investigated the intermediate products formed in the nitrification of ammonium salts. He carried the experiments in filters inoculated from actively nitrifying sewage filters. The compounds identified were hydroxylamine salts and salts of hyponitrous and nitrous acids. As he used only a mixed culture from a sewage, it cannot be concluded from these results that hydroxylamine and hyponitrous acid will be formed in the normal course of oxidation of ammonia by the organism nitrosomonas. Beesley (*J. Chem. Soc.*, 1914, **105**, 114) examined the rate of nitrification of urea, uric acid, asparagine, glycine, methylamine, acetamide, ammonium oxalate, and ammonium sulphate. He employed dilute solutions of the various compounds in the presence of suitable mineral nutrients and inoculated them with a mixed culture from a sewage filter. He inferred that possibly hydroxylamine and hyponitrous acids are formed as intermediate products. His experiments appear to be inconclusive, as he used a mixed culture containing many different types of organisms—this is evident from the fact that he obtained nitrification of complex nitrogenous compounds like asparagine, urea, etc., though nitrosomonas in pure culture is without action on such compounds. Maze (*compt. rend.*, 1921, **172**, 173) thought that hydroxylamine is an intermediate stage in the oxidation of ammonia to nitrite. Kluyver and Donker (*Chem. Zelle. Gewede*, 1926, **13**, 134) tacitly assume the possible formation of hydroxylamine and hyponitrous acid. Corbet (*Biochem. J.*, 1934, **28**, 1575; 1935, **29**, 1086) stated that he could identify hydroxylamine on occasions and hyponitrous acid frequently in nitrifying cultures. He has also estimated the amount of the hyponitrite formed. Nelson (*Centl. Bakt.*, 1931, *ii*, **83**, 280) reported his failure to detect any hydroxylamine. Gopal Rao and Pandalai (*loc. cit.*) examined a very large number of nitrifying cultures at different stages and failed to detect hydroxylamine.

From the theoretical standpoint, the question of the formation of intermediate products is an important one. The Warburg theory of biological oxidations assumes activation of molecular oxygen by the "atmungsferment" present on the cell surface: then follows the oxidation of the substrate molecule by the activated oxygen. On the other hand, Wieland assumes that activation of the substrate molecule is more important than the activation of oxygen. He considers that the substrate molecule is activated in such a way that it can easily part with one or more molecules of hydrogen, which will subsequently combine with a suitable hydrogen acceptor like oxygen, methylene blue, etc. Thus according to Wieland, oxidation is

in essence a dehydrogenation—he has shown that many familiar cases of oxidation which are usually regarded to involve addition of oxygen are in reality cases of dehydrogenation, such oxidations being brought about in the absence of oxygen but in the presence of suitable hydrogen acceptors like methylene blue, nitrobenzene, quinone, etc. Progressive dehydrogenation may result in the formation of intermediate products. For instance, in the oxidation of ethyl alcohol to acetic acid by the *bacterium aceti* Wieland showed that acetaldehyde formation is an intermediate stage; and that the bacterium can oxidise acetaldehyde to acetic acid. This discovery lends considerable strength to Wieland's theory that most oxidations are dehydrogenations.

The following scheme represents the progressive dehydrogenation of ammonia in aqueous solution. It is well known that the nitrifying organisms bring about the oxidation in a medium that is definitely alkaline. The optimum p_H is about 8·4—a solution of ammonium sulphate is not acted upon as such ; the presence of a mild base like calcium carbonate or magnesium carbonate is found necessary. It is clear that it is the ammonium hydroxide and not the ammonium ion that is involved.



or

Thus in the progressive dehydrogenation of ammonium hydroxide one expects hydroxylamine and hyponitrous acid as intermediaries. In view of the importance of the problem it was thought desirable to investigate this aspect carefully.

EXPERIMENTAL.

For the culture of the nitrite forming organisms, the following medium was utilised.

Ammonium sulphate	... ...	2·0 g.
Sodium chloride	... ...	2·0
Dipotassium hydrogen phosphate	... ...	1·0
Magnesium sulphate	... ...	0·5
Ferrous sulphate	... ...	0·4
Sterile distilled water	... ...	1000 c.c.

Portions (100 c.c.) of this medium were poured into a sterile culture flaks of 500 c.c. capacity and sterilized. Sterile magnesium carbonate (1 g.) was then added to the solution. This was seeded with one gramme of garden soil. The flask was then plugged with sterile cotton and incubated at 30°. Nitrite at first developed slowly and then more rapidly during the later days of incubation. When nitrite was developed appreciably, as shown by the Griess-Ilosva reaction, a few drops of the culture solution were introduced by means of sterile platinum loop or sterile pipette into another sterile flask containing 100 c.c. of the fresh medium and 1 g. of magnesium carbonate. In this manner several transfers were made, each time before ammonia disappears completely. Thus by this method the nitrate former is eliminated and a fairly pure culture of the nitrite former is obtained; by this process of elective culture, other soil organisms are also eliminated

Hydroxylamine.—The tests employed for the detection of hydroxylamine are those of (*i*) W. C. Ball, (2) Fischer (+ or 1 and 2—*vide* J. W. Mellor "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Vol. VIII, p. 295) and of J. Blom (*Ber.*, 1926, **59**, 121) as also those depending on the reduction of mercuric oxide and copper oxide. In many hundreds of cultures examined, hydroxylamine could not be detected. Our results are in agreement with those of Nelson (*loc. cit.*). The cultures were prepared from different soils, those from Waltair, Vizagapatam, Coimbatore, Raipur and Pusa. J. Blom has stated that the addition of acetone in small amounts (0·4 c.c. per litre) served the purpose of combining with the hydroxylamine and storing it. Even in this way we could not obtain the test.

Hyponitrites.—Corbet (*loc. cit.*) reported to have discovered a new and delicate test for hyponitrites: The reagent suggested is a mixture of equal volumes of a saturated solution of potassium periodate and a solution of resorcinol. This is said to give a cherry-red colour with a neutral or slightly acid solution of hyponitrite.

We have now carried out systematic work on the utility of this colour test. The reagent we employed was a mixture of equal volumes of an $M/5$ solution of resorcinol and saturated solution of potassium periodate. The mixture was prepared immediately before use, as it darkens on standing. We find that the nature and intensity of the colour depend on the H^+ ion concentration of the test solution. Reliable results are obtained at p_H 2 or p_H 3. To a buffer solution made up of 7 c.c. of $N/10$-hydrochloric acid, and 3 c.c. of sodium citrate solution, 1 c.c. of the neutral test solution is added, followed by the addition of 2 c.c. of the resorcinol potassium periodate reagent. In the presence of hydroxylamine, hyponitrite and nitrite, a cherry-red colour is obtained in five to 15 minutes, the intensity of the colour depending on the amount of the substance present. At various H^+ ion concentrations, the above three substances behave similarly and we are to conclude that the test is not specific for hyponitrite. The test is sensitive down to 1 part of nitrite or hydroxylamine or hyponitrite in a million part of water.

In nitrifying cultures, nitrite is formed in increasing amounts and hence the above test will not be of any use to detect the formation of hyponitrite. The findings of corbet that hyponitrite is detected by the above test are thus open to criticism. Corbet thought that the above test is specific for hyponitrite. This is not the case. Further work is in progress.

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THE DETERMINATION OF THE PARACHORS OF INORGANIC SALTS IN SOLUTIONS. PART III. THE PARACHORS OF SOME SALTS OF MAGNESIUM, STRONTIUM AND BARIUM, AND THE ATOMIC PARACHOR OF THE ABOVE ELEMENTS AND RADIUM.

By Jamiat V. Lakhani and Rustom P. Daroga.

The " solution method " of measuring parachor has been extended to determine experimentally the parachors of some Mg, Sr and Ba salts, not previously done. The atomic parachor of Mg and Sr (previously not known) has been computed from the data of the salts. The log atomic number—log parachor relationship has been found to hold in the case of group II metals also and a straight line curve is obtained. The atomic parachor of radium from the above relationship is deduced to be 140.

The object of the present investigation has been to ascertain whether the log atomic number—log parachor relationship that has been established by the authors in the case of the alkali metals (*J. Indian Chem. Soc.*, 1938, **15**, 519) would also hold in the case of alkaline earth metals (Be, Mg, Ca, Sr, Ba) and if so to deduce therefrom the atomic parachor of radium, which belongs to the same group of elements but whose parachor cannot be determined on account of the high cost and rarity of the metal and its salts. The parachor of beryllium and calcium has been known by the fusion method with a degree of certainty (Sugden, " Parachor and Valency " pp. 145, 187) but that of magnesium, strontium and barium atoms has not been calculated or experimentally established. In consequence of this, the parachors of some soluble salts of magnesium, strontium and barium have been determined by the " solution method " (*J. Indian Chem. Soc.*, 1938, **15**, 37) and the atomic parachors of the above metals have been deduced from the parachors of the salts. This deduction is based on the fact pointed out by the authors (*loc. cit.*) that the parachor is invariably found to maintain its additive character in the case of simple inorganic salts.

Experimental.

All measurements of surface tensions have been carried out at 30 + ·02° by the capillary rise method and the apparatus and other experimental details were exactly the same as already described (*J. Indian Chem. Soc.*, 1938, **15**, 37).

In the table below are given the data and the results obtained with three magnesium salts, three strontium salts, and two barium salts.

TABLE I.

Magnesium, strontium and barium salts.

Substance.	X.	D.	V.	M_m.	P_m.	P_x (parachor observed).	$[P]$ without metal.	Parachor of the metal deduced from the observed value of the salt.
$MgCl_2, 6H_2O$ (temp., 30·2°)	0·03117	1·125	83·43	20·408	54·84	161·7		Mg = 57·2
	0·03634	1·144	84·14	20·811	55·08	163·5		
						(mean 162·6)		
$Mg(NO_3)_2$, $6H_2O$ (temp., 35°)	0·02283	1·129	79·50	20·977	55·49	238·2		Mg = 56·7
	0·02379	1·133	79·58	21·098	55·55	238·8		
						(mean 238·5)		
$MgSO_4, 7H_2O$ (temp., 35°)	0·02082	1·128	77·56	20·135	52·95	177·62		Mg = 59·12 (mean 57·7)
$SrCl_2$, $6H_2O$	0·02273	1·165	82·85	21·194	54·91	188·34	105·4	85·76
	0·02732	1·199	84·31	21·843	55·18	193·98		
						(mean 191·16)		
$SrBr_2$, $6H_2O$	0·03004	1·335	79·00	24·893	55·59	214·4	132·8	83·6
	0·03157	1·351	79·12	25·251	55·75	218·4		
						(mean 216·4)		
$Sr(NO_3)_2$	0·03292	1·271	75·96	24·375	56·61	266·8	181·8	85·0 (mean 85·78)
$BaCl_2$ (anhydrous)	0·02164	1·172	73·01	22·112	55·03	212·5	105·4	107·2
$Ba(NO_3)_2$ (temp., 60°)	0·01240	1·138	69·44	21·021	53·31	287·0	181·8	105·2 (mean 106·2)

It may be noticed (*a*) that the parachor of the above mentioned magnesium and strontium salts or the two metals has not been previously determined on account of the difficulty of fusing them at high temperature.

(*b*) That in the case of $Ba(NO_3)_2$, the salt not being sufficiently soluble at the temperature of 30° to give the required molar fraction for parachor determination, the surface tension and specific gravity have been determined at 60° at which temperature the solubility of this salt is sufficiently high.

(*c*) That the slightly divergent values obtained with different molar fractions of the strontium salts are due to slight changes in the volume that take place when different quantities of the salts are dissolved in the same quantity of water.

(*d*) That the mean parachor value of magnesium atom is deduced to be 57·7 and that of strontium as 85.

The parachor of barium calculated from the surface tension and density data of fused $BaCl_2$ obtained by Motylewski (*Z. anorg. Chem.*, 1904, **38**, 410) is found to be 106. Sugden ("Parachor and Valency", p. 117) has regarded this figure as a rough value in error by ±10 units. The parachor of barium deduced from its salts (Table I) is found to be 106·2, and as this figure is confirmed from Motylewski's data, it may reasonably be taken as the more probable value of the parachor of barium atom.

In Table II below, are given the data of atomic numbers and parachors of the elements of group II and also the log values of the constants. In the table below are given the data for the two graphs.

TABLE II.

Element.	Symbol.	Atomic No. $=X$.	Parachor $=Y$.	Log X.	Log Y.
Beryllium (Sugden, "Parachor and Valency", p. 145)	Be	4	37·8	0·6021	1·5775
Magnesium	Mg	12	57·7	1·0792	1·7612
Calcium (Motylewski, *loc. cit.* Sugden, *loc. cit*)	Ca	20	68	1·3010	1·8325
Strontium	Sr	38	85	1·5798	1·9294
Barium	Ba	56	106	1·7482	2 0253
Radium	Ra	88	140 (value deduced from the curve)	1 9445	2 146 (value read on the curve)

FIG. 1*a*.

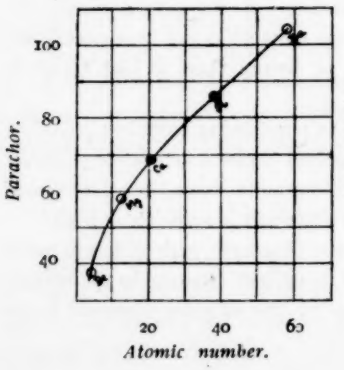
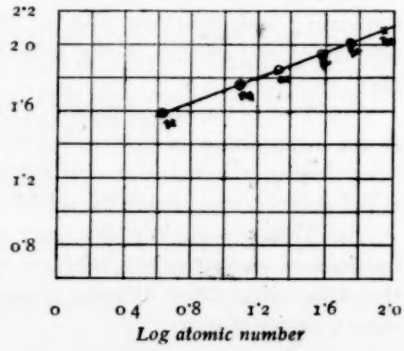


FIG. 1*b*.



The curve in Fig. 1*a* is obtained when the parachor of each element is plotted against its atomic number, and the curve in Fig. 1*b*, which is a straight line, is obtained when the log values of the two constants are plotted. When the straight line is extrapolated, the parachor of radium comes to be 140. As mentioned above, there is no direct means available at present for ascertaining the value experimentally. But it is clear from the straight line curve (Fig. 1*b*), that the log atomic number—log parachor relationship holds in the case of alkaline earth metals and that the parachor is intimately connected to the fundamental property of the atom and the atomic number. Consequently not only the values of atomic parachor of magnesium, strontium, and barium computed from their salts may be taken as reasonably correct but that it is also justifiable to deduce the parachor of radium in the manner it has been done.

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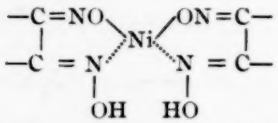
OXALENEDIAMIDOXIME. PART I. ESTIMATION OF NICKEL.

By R. Chatterjee.

Oxalenediamidoxime has been used as an analytical reagent for the estimation of nickel up to 0·006 g., and for its separation from zinc and manganese. Quantitative separation from cobalt has been found possible within narrow limits of concentrations of cobalt.

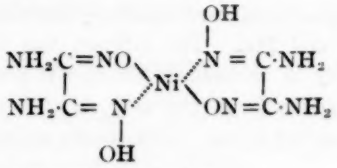
It is well known that the atomic grouping $HON{=}\overset{|}{C}{-}\overset{|}{C}{=}NOH$ reacts specifically with the nickel ion in a characteristic fashion to form inner metallic complexes of the type



The monobasicity of dioximes obviously lowers the percentage content of nickel in the complex, and the simultaneous saturation of primary and co-ordination valences by chelation lowers the solubility of such complexes in highly polar solvents like water, both of which effects are favourable for the utilisation of such organic molecules as analytical reagents.

It may be pointed out that the best known compound among this class of dioximes, *viz.*, dimethylglyoxime, has the disadvantage of not being quite soluble in aqueous media from which nickel is precipitated, and that the complex itself has fair solubilities in both acids and ammonia. A similar dioxime possessing, however, greater solubility in water, has been found in oxalenediamidoxime, and the possibility of using this as a precipitant for nickel has been indicated here. It may be seen from the results obtained that, leaving perhaps the important application of dimethylglyoxime as a reagent for the separation of nickel from cobalt, this diaminodioxime serves quite a satisfactory reagent for the individual estimation of nickel, and in its separation from other metals of group IIIB of the analytical table. The slow, incomplete precipitation of cobalt is doubtless connected with the amine character of the reagent (Feigl and Christiani-Kronwald, *Z. anal. Chem.*, 1925, **65**, 341), but the dark brown, powdery compound appears to the present author to be more complex than the simple cobalt analogue of the nickel dioxime compound.

The constitutional formula of the nickel complex is tentatively given as



in analogy with the dimethylglyoxime complex.

Experimental.

Preparation of the Reagent.—Fischer's method of preparation was followed (*Ber.*, 1889, **22**, 3930). A rapid stream of cyanogen gas was passed into a concentrated, ice-cold, aqueous solution of hydroxylamine hydrochloride (1 mol.) and caustic potash (1 mol.). Oxalenediamidoxime separated in colourless crystals, and was purified by recrystallisation from hot water (m.p. lit. 200° with decomp., found, 199° with decomposition). The reagent is readily soluble in hot water ; a dilute solution of the reagent was used in the estimations.

Nickel Oxalenediamidoxime.—The light orange, crystalline precipitate of the nickel complex, obtained by precipitating with a dilute solution of the reagent from faintly ammoniacal solution of nickel chloride containing ammonium chloride, was washed with cold water, and readily obtained anhydrous by drying at 110-120° for half an hour. [Found : Ni (by ignition to oxide), 20·02. Calc. for $Ni(C_2H_5N_4O_2)_2$: Ni, 20·04 per cent].

Estimation of Nickel.—A solution of chemically pure nickel chloride containing about 0·2% nickel was prepared and standardised by the dimethylglyoxime method. Certified standard pipettes were used for measuring out known volumes of nickel solution. To the nickel solution at room temperature was added a cold dilute solution of the reagent in slight excess, and then ammonium hydroxide drop by drop with stirring, till the solution smells of ammonia. The light orange precipitate of the nickel complex was allowed to settle, filtered through asbestos filter, washed with cold water, dried at 110-120°, and weighed. Table I shows the results.

Table I.

Wt. of Ni oxime ppt.	Weight of nickel found.	Weight of nickel taken.	Wt. of Ni oxime ppt.	Weight of nickel found.	Weight of nickel taken.
0·0297 g.	0·00595 g.	0·00598 g.	0·1116 g.	0·0224 g.	0·0224 g.
0·0596	0·01194	0·01195	0·1120	0·0224	0·0224
0·1193	0·0239	0·0239	0·1111	0·0223	0·0224

Separation of Nickel from Zinc and Manganese.—A known volume of the standard nickel solution was mixed respectively with known volumes of zinc and manganous chloride solutions, containing approximately known amounts of Zn and Mn. The mixture was in each case treated with a sufficient quantity of ammonium chloride to prevent precipitation of the hydroxides in ammoniacal medium. Nickel was then precipitated and estimated as described above. The results are shown in Table II.

TABLE II.

Composition of soln.		Wt. of ppt.	Wt. of Ni found.	Error.
0·0224 g. Ni + 0·025 g. Zn		0·1112 g.	0·0223 g.	−0·0001
0·0239	0·025	0·1190	0·0238	−0·0001
0·0239	0·05	0·1193	0·0239	Nil
0·0239	0·125	0·1196	0·0240	+0·0001
0·0239	0·25	0·1192	0·0239	Nil
0·0239	0·375	0·1191	0·0239	Nil
0·0224	0·014 Mn	0·1117	0·0224	Nil
0·0239	0·024	0·1194	0·0239	Nil
0·0239	0·048	0·1196	0·0240	+0·0001
0·0239	0·24	0·1196	0·0240	+0·0001
0·0239	0·36	0·1193	0·0239	Nil

Separation of Nickel from Cobalt.—A dark brown, insoluble powder slowly separated from ammoniacal solutions containing cobalt on the addition of the reagent. The precipitation was found to be accelerated by passing a current of air, and temporarily prevented by the addition of hydroxylamine hydrochloride. The estimation of nickel in presence of cobalt was carried out in exactly the same manner as in other cases, with the only difference that nearly 1 g. of hydroxylamine hydrochloride was added for every 100 c.c. of the solution, before rendering the sclution ammoniacal. Some of the results are shown in the following table. Larger amounts of cobalt than those shown here led to high results for nickel.

TABLE III.

Composition of soln.		Wt. of ppt.	Wt. of Ni found.	Error.
0·0239 g. Ni + 0·0125 g. Co		0·1199 g.	0·0240 g.	+0·0001
0·0239	0·025	0·1190	0·0238	−0·0001
0·0239	0·05	0·1193	0·0239	Nil

The author is indebted to the very Rev. A. Schockert S. J., Rector of the College, for providing all facilities for work.

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Received November 3, 1933.

ON THE ESTIMATION OF FUMARIC AND MALEIC ACIDS.

By S. C. Ganguly.

It has been shown that fumaric and maleic acids can be estimated in acid solution, in presence of succinic acid and phosphates with mercuric sulphate as catalyser.

In connection with a work in this laboratory it was necessary to estimate small amounts of fumaric acid in presence of succinic acid and phosphate buffers. Estimation by $KMnO_4$, either in acid or in alkaline solution as proposed by Straub (*Z. physiol. Chem.*, 1935, **1**, 236) and Strumm (*Z. angew. Chem.*, 1937, **47**, 791), were not suitable because (*i*) $KMnO_4$ oxidises fumaric as well as succinic acid and (*ii*) the reaction between $KMnO_4$ and fumaric acid is not stoichiometric, but increases with the added amount of $KMnO_4$ with no definite end-point. The three second end-point suggested by Straub was found not to give good results.

McMargasches and Hinner (*Z. anal. Chem.*, 1924, **64**, 61) suggested the possibility of estimating fumaric acid by bromination in nearly neutral solutions. This method was studied extensively by Szegedy (*Z. anal. Chem.*, 1937, **109**, 95). He has shown that the p_H value of the solution must be controlled at p_H 8·4, otherwise erratic values would be obtained. Moreover at a p_H value above the transition point of phenolphthalein, the bromination of succinic acid is not nil. The direct halogenometric estimation of fumaric acid in presence of phosphates at p_H 9·4 gives high values (Szegedy, *Z. anal. Chem.*, 1937, **109**, 316). Hence Szegedy recommends that when phosphates are present in the solution, fumaric acid should be previously separated as mercurifumarate. The objection to the estimation of fumaric acid in acid solution seems to be due to the slowness of the reaction. In this connection Frieman, Kennedy and Lucas (*J. Amer. Chem. Soc.*, 1937, **59**, 722) have shown that the direct estimation of acetylenes can be carried out by direct bromination in presence of $HgSO_4$ as catalyst. This method has been developed by Lucas and Pressmann (*Ind. Eng. Chem. Anal. Ed.*, 1935, **10**, 149) and a comprehensive scheme has been drawn up for the estimation of various unsaturated compounds by the bromate-bromide mixture, amongst which have been included maleic and fumaric acids. But no experiment was carried out by them in presence of succinic acid and phosphates. In view of the long time required for the complete bromination of maleic and fumaric acids in acid solution, even in presence of $HgSO_4$ (30-35 mins.), the elucidation of the effect of these two substances was thought desirable.

Experimental.

The method of titration was similar to that described by Lucas and Pressman (*loc. cit.*).

A known excess of bromate-bromide mixiure (10 to 15%) was taken in the titration vessel and the flask was evacuated. 5 C.c. of $6N$-H_2SO_4 were added and allowed to stand for 2-3 minutes, while the bromine was being liberated. The flask was then wrapped with a black cloth and the respective quantities of $0{\cdot}2N$-$HgSO_4$ and the solution to be analysed were run in without breaking the vacuum. The wash-liquid in no case exceeded 10% of the total volume. The flask was then shaken well and allowed to stand in the dark for 30 to 35 minutes. $2N$-NaCl solution (15 c.c.) was then added to liberate the bromine and then 5 to 10 c.c. of 20% KI. The flask was shaken for $\frac{1}{4}$ minute, the vacuum broken, and the liberated iodine was then titrated by $N/50$-Hypo solutions. All the reactions were carried out at room temperature.

Titration of Fumaric Acid alone.

Table I.

Effect of change of $HgSO_4$.

1·15 C.c. $N/10$-$KBrO_3$ + 5 c.c. $6N$-H_2SO_4 + $0{\cdot}2N$-$HgSO_4$ + 10 c.c. fumaric acid solution containing $0{\cdot}00584N$-acid allowed to stand for 40 minutes ; 5 c.c. of $2N$-NaCl added. Back titrated with KI and hypo.

$0{\cdot}2N$-$HgSO_4$	$KBrO_3$ consumed.		$0{\cdot}2N$-$HgSO_4$.	$KBrO_3$ consumed.	
	Actual.	Theoretical.		Actual	Theoretical.
5 c.c.	0·992 c.c.	1·00 c.c.	10 c.c	0·980 c.c.	1·00 c.c.
8	1·02	,,	15	1·00	,,

Table II.

Effect of variation of time.

1·15 C.c. $N/10$-$KBrO_3$ + 5 c.c. $6N$-H_2SO_4 + 5 c.c. $0{\cdot}2N$-$HgSO_4$ + 10 c.c. of fumaric acid.

Time.	$KBrO_3$ consumed.		Time.	$KBrO_3$ consumed.	
	Actual.	Theoretical.		Actual	Theoretical.
10 min.	0·750 c.c.	1·00 c.c.	30 min.	1·04 c.c.	1 00 c.c.
20	0·975	,,	40	0·992	,,

TABLE III.

Titration of fumaric acid in presence of phosphates.

1·15 C c. $N/10$-$KBrO_3$ + 5 c.c. $6N$-H_2SO_4 + 0·2N-$HgSO_4$ + 5 c.c. of $M/15$-Na_2HPO_4 + 10 c.c. of fumaric acid. Reaction allowed to continue for 30 mins.

$HgSO_4$.	$KBrO_3$ consumed.		$HgSO_4$.	$KBrO_3$ consumed.	
	Actual.	Theoretical.		Actual.	Theoretical.
7·5 c.c.	0·900 c.c.	1·00 c.c.	15·0 c.c.	0·995 c.c.	1·00 c.c.
10·0	0·950	,,	20·0	1·01	,,
			25·0	0·987	,,

Effect of Succinic Acid on the Estimation of Fumaric Acid in presence and absence of Phosphates.

TABLE IV.

Effect of bromate mixiure on succinic acid alone.

3·0 C.c. $N/10$-$KBrO_3$ + 10 c.c. $HgSO_4$ + 5 c.c. $6N$-H_2SO_4 + 10 c.c. 0·05N succinic acid.

Time.	$KBrO_3$ consumed (from back titration bv Hypo).	Time.	$KBrO_3$ consumed (From back titration by Hypo).
* 10 mins.	Nil	40 mins.	Nil
20	,,	35	0·01 c.c.
		40	Nil

TABLE V.

Estimation of fumaric acid solution containing succinic acid and phosphates..

1·15 C.c. $N/10$-$KBrO_3$ + 5 c. c. $6N$-H_2SO_4 + 15 c.c. 0·2 N-$HgSO_4$. The reaction allowed to continue for 30 mins.

$N/20$-Succinic acid.	$N/300$-Fumaric acid.	$M/15$-NH_2HPO_4.	$N/10$-$KBrO_3$ consumed. Obs.	Theor.
5 c.c.	10·0 c.c.	...	0·985 c.c.	1·00 c.c.
10·0		...	1·02	,,
15·0		...	1·03	,,
5·0		5·0 c.c.	0·980	,,
20·0	*5·0	,,	0·505	0·50
20·0	*3·0	,,	0·295	0·30
20·0	*1·0	,,	0·110	0·10

* In cases marked with asterisk, $KBrO_3$ solution added was from 0·55 c.c. to 0·125 c.c.

Estimation of Maleic Acid in presence of Succinic Acid and Phosphates.

TABLE VI.

Conditions exactly the same as in the estimation of fumaric acid.

$N/10$-Succinic acid.	$N/100$-Maleic acid.	$M/15$-Na_2HPO_4.	$KBrO_3$ consumed. Obs.	Theor.
10·0 c.c.	10·0 c.c.	5·0 c.c.	1·02 c.c.	1·00 c.c.
10·0	15·0	5·0	1·485	1·50
10·0	20·0	5·0	1·980	2·00
10·0	25·0	5·0	2·50	2·50

Comparison of Szegedy's Method and the Mercuric Sulphate Method in Estimating Fumaric Acid from a Solution containing $M/15$- phosphate.

TABLE VII.

Fumaric acid actually taken.	Obs. value by Szegedy's method after separation as mercurofumarate.	Obs. value by mercuric sulphate method.
0·00584 g.	0·00582 g.	0·00580 g.
0·1168	0·1145	0·1170
0·01752	0·01750	0·01748

It will be observed that whereas at p_H 8·4, high values were obtained by Szegedy in the direct halogenometric titration of fumaric acid in presence of phosphates, low values were obtained when fumaric acid was titrated alone in acid solution in presence of phosphates. It seems probable that whereas HPO_4'' and H_2PO_4' are capable of catalysing the bromination of fumaric acid, undissociated H_3PO_4 probably retards the bromination.

Further work is in progress in this direction.

My best thanks are due to Prof. N. N. Sen for kind interests and encouragement.

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NOTE ON OXIDATION OF FERROUS IRON WITH POTASSIUM IODATE.

By Balwant Singh.

Fe" has been estimated potentiometrically against KIO_3 in presence of an excess of HCl and in an atmosphere of CO_2.

Fe is iron has been determined potentiometrically by titrating it against standard potassium iodate in presence of an excess of hydrochloric acid in an atmosphere of carbon dioxide.

A known weight of ferrous ammonium sulphate was taken in the titration vessel, from which the air was swept off by a current of carbon dioxide and a required amount of hydrochloric acid was added to keep its concentration above $4N$. Standard potassium iodate was then added from a burette, the mixture was stirred by a mechanical stirrer and the progress of oxidation followed with the potentiometer.

In these titrations, with the addition of standard potassium iodate the E. M. F. rose steadily till the equivalence-point. At the equivalence-point, there was a sharp jump in potentials in each case. For the addition of 0·05 c.c. of the titrant, the inflection potential was of the order of 220 millivolts. After the equivalence-point, there was again a rise in the potential which became steady on further addition of the reagent.

From the volume of the potassium iodate solution, required in each titration corresponding to the equivalence-point, the amount of the salt was calculated. The values obtained are compared with the amounts of the salt taken in the table given below.

Table I.

Ferrous ammonium sulphate.

Taken.	Found.	Taken.	Found.
0·7830 g.	0·7827 g.	0·3788 g.	0·3784 g.
0·6215	0·6211	0·2397	0·2395
0·5174	0·5172	0·1190	0·1187
		0·0580	0·0576

The author thanks the Khalsa College authorities for a research grant which has enabled him to carry out this work.

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OBITUARY

NARHAR WAMAN HIRWE, B.A., M.Sc., A.I.C.

Born March, 28, 1898. Died August 11, 1938.

Narhar Waman Hirwe who died on 11th August, 1938 was a Fellow of the Society since 1927 and Honorray Treasurer of the Bombay Branch for the last three years. He was in indifferent health for nearly two years and died almost a week after an operation for appendicitis.

He was born on 28th March, 1898 at Belapur in the Ahmednagar district. He received his early education at the place of his birth and then at Sangamner from where he went to Poona to join the New English School. He received the collegiate education at the Ferguson College, Poona passing the B.A. and the B.Sc. examinations in 1921 and 1923 respectiveiy. In June 1923 he joined the Royal Institute of Science, Bombay to work under the guidance of the late Dr. A. N. Meldrum. He was appointed a Fellow of the Institute for one year during 1924-25. He was awarded the M.Sc. degree of the University of Bombay in 1926 with distinction for his thesis on the nitrosalicylic acids.

After working for a short time as a lecturer in Chemistry at the Wilson College, Bombay, he joined the Royal Institute of Science as an Assistant Lecturer in the Department of Organic Chemistry where he continued to work until a week before his death.

His first research paper was published with Dr. Meldrum in 1928 in the Journal of the Society on the nitrosalicylic acid followed by another in 1930 on the same subject. He continued his investigations on this problem and published, till the time of his death, twelve papers entitled " Derivatives of salicylic acid " and one on the " Studies in chloral derivatives of salicyl amides." He was also associated with Dr. Jadhav in two papers on the " Derivatives of 1-hydroxy-2-naphthoic acid."

He leaves behind him his widow, three daughters, a son and a large circle of students and friends to mourn his loss. May his soul rest in peace !

S. M. M.

THE VITAMIN B COMPLEX IN TOXIC CONDITIONS.

By A. Mukherji.

The effects of aneurin, lactoflavin and frankonite filtrate of aqueous yeast extract in toxic conditions caused by 15% codliver oil in foods of a colony of white rats weighing 50—60 g. each, have been tried All of them cause detoxication but lactoflavin seems to be most effective. Vitamin B complex acts better than any of the factors alone.

Agdhur (*Acta Pediatri*, 1926, **5**, 319 ; 1926, **6**, 165 ; 1928, **7**, 289 ; 1928, **8**, 364 ; 1929, **8**, 489 ; 1929, **9** 170) had found that large amount of codliver oil given to animals produced toxic effects, which were curable with large amounts of yeast. Norris and Church (*J. Biol. Chem.*, 1930, 437) had also found the same thing and it appears from their work that aneurin is the factor concerned in preventing the toxic conditions. De Vries and Puister (*Ext. Archiv. Néerl. Physiol.*, 1933, **18**, 71) also worked on the same problem and they are of opinion that the vitamin B_2 is the factor in the yeast which cures the toxic conditions caused by the administration of large amounts of codliver oil. But in none of their experiments they have used the pure vitamins aneurin and B_2. Sometime back Coward and her co-workers ("The biological Standardisation of the Vitamins," London, 1938) had shown to some extent that the different factors of the vitamin B complex are to some degree replaceable by the other factors of the same vitamin (using growth effect as the criterion). Mukherji (" The Role of Aneurin-vitamin B_1 and Vitamin D in Guanidine and Methylguanidine poisoning", Amsterdam University, 1938, p. 8) had also found the same thing from another aspect. He had shown that the aneurin neutralises the toxic effects of the guanidine bodies but if the vitamin B complex was used instead of the aneurin alone then the amount of aneurin required to neutralise the effect of the same amount of guanidine was much less than before. Hariette Chick (*Lancet*, 1933, **2**, 341) had found that pellagra is caused by a toxic substance derived from a corn diet which effect could be corrected by a water-soluble substance associated with the vitamin B_2. It was thought desirable at this stage to find out individually the effects of the different factors of the vitamin B complex in neutralising the toxic effects of the same substance. With this end in view the present work was undertaken with large quantities of codliver oil and the different factors of the vitamin B complex in a chemically pure state wherever they are available. It is found that the vitamins aneurin, lactoflavin and a heat-labile factor in the B_n complex (=vit. B complex − vit. B 1 and 2) are potent detoxicating agents ; the latter two factors being again more potent than aneurin in neutralising the toxic effects of large amounts of codliver oil.

EXPERIMENTAL.

The animals used in this work were young white rats weighing about 50 g. each. They were taken from the same colony. Aneurin used was prepared by Hoffmann (La Roche, Basle) and the vitamin B_2 (lactoflavin) in the Laboratory of Physiological Chemistry, University of Amsterdam, both of them being synthetically prepared pure crystals. Aneurin (0·1 mg.) was injected and lactoflavin (0·05 mg.) daily to those animals receiving them by the intraperitoneal route, Sundays and some holidays being excepted. Other factors of the vitamin B complex were used as dried whole yeast, autoclaved yeast and frankonite filtrate from aqueous extract of yeast. Nicotinic acid was also tried. All these were administered, mixed with food. It has been found that better results were obtained if the foods were prepared fresh about twice a week, specially during the summer days. Otherwise it has been found that foods get more toxic and the results are vitiated unexpectedly. In foods Mu 1, 2, 5 and 16, 3% autoclaved yeast were used instead of whole yeast. In each case the total amount of washed rice, oil, and yeast was kept constant. Mu 1 and 7 had daily 0·1 mg. aneurin; Mu 2 and 12, 0·05 mg. lactoflavin intraperitoneally, Mu 8 had frankonite filtrate from aqueous extract of 200 g. of yeast per kg. of the food. Composition of foods is shown in Table I.

TABLE I.

Composition table of diets used (in per cent.).

Food.	Rice.	Casein.	Salts.	Yeast.	Codliver oil.	Addenda.
Mu 1	74	5	3	3 (autoclaved)	15	Aneurin
Mu 2	74	5	3	3 ,,	15	Lactoflavin
Mu 5	73	5	3	3 ,,	1	14% Peanut oil
Mu 6	74	5	3	3 (whole dried)	1	14% Peanut oil
Mu 7	74	5	3	3 ,,	15	Aneurin
Mu 8	74	5	3	3 ,,	15	Frankonite filtrate
Mu 9	74	5	3	3 ,,	15	Nicotinic acid (2 g.)
Mu 10	62	5	3	15 ,,	15	
Mu 12	74	5	3	3 ,,	15	Lactoflavin
Mu 13	81	5	3	3 ,,	8	
Mu 14	81	5	3	3 ,,	8	Aneurin
Mu 15	69	5	3	15 ,,	8	
Mu 16	74	5	3	3 (autoclaved)	15	Aneurin and lactoflavin

The results of the feeding experiments are shown in the following tables.

TABLE II.

Rat No.	Weight on 25-43-8.	9-5-38.	Max increase.	Food.	Rat No.	Weight on 25-4-38.	9-5-38.	Max. increase.	Food.
8995	58 g.	40 g.	−18 g.	Mu 1	9001	58 g.	40 g.	−18 g.	Mu 2
8996	57	40	−17	1	9002	59	40	−19	2
8997	59	42	−17	1	9003	62	96	34	5
8998	57	39	−18	1	9015	50	83	33	5
8999	60	46	−14	2	9016	47	78	31	5
9000	54	38	−16	2	9017	51	93	42	5

TABLE III.

Rat No.	Weight on 1-5-38.	19 7-38.	Max. increase.	Food.	Rat No.	Weight on 19-5-38.	1-7-38.	Max. increase.	Food.
8995	54 g.	67 g	27 g.	Mu 7	9001	58 g.	46 g.	18 g.	Mu 12
8996	57	79	39	7	9002	63	45	23	12
8997	64	87	45	7	9003	124	171	75	6
8999	54	76	37	7	9015	105	183	100	6
8998	71	49	25	12	9016	98	163	85	6
9000	57	46	19	12	9017	116	197	104	6

TABLE IV.

Rat No.	Weight on 31-5-38.	1-7-38.	Max. increase.	Food.	Rat No.	Weight on 31-5-38.	1-7-38.	Max. increase.	Food.
9291	32 g.	62 g.	30 g.	Mu 7	9297	35 g.	77 g.	42 g.	Mu 8
9292	41	74	33	7	9298	28	58	30	8
9293	42	71	29	7	9299	29	65	36	8
9294	45	58	13	7	9300	26	53	27	8
9295	42	70	28	7	9301	29	51	22	8
9296	39	58	19	7	9302	30	57	27	8

TABLE V.

Rat No.	Weight on 9-5-38.	1-7-38.	Max. increase.	Food.	Rat No.	Weight on 9-5-38.	1-7-38.	Max. increase.	Food.
9042	64 g.	116 g.	52 g.	Mu 8	9048	46 g.	38 g.	−8 g.	Mu 9
9043	41	95	54	8	9049	46	40	−8	9
9044	60	117	57	8	9050	55	121	66	10
9045	57	110	53	8	9051	53	108	55	10
9046	46	38	−10	9	9052	54	102	48	10
9047	44	38	− 9	9	9053	46	104	58	10

TABLE VI.

Rat No.	Weight on 4-5-38.	1-7-38.	Max. increase.	Food.	Rat No.	Weight on 4-5-38.	1-7-38.	Max. increase.	Food.
9054	40 g.	112 g.	72 g.	Mu 13	9060	40 g.	107 g.	67 g.	Mu 14
9055	42	107	65	13	9061	41	116	75	14
9056	45	147	102	13	9062	42	221	179	15
9057	33	113	80	13	9063	42	168	127	15
9058	41	113	72	14	9064	46	183	137	15
9059	45	118	73	14	9065	33	116	83	15

TABLE VII.

Rat No.	Weight on 23-6-38.	18-7-38.	Max. increase.	Food.	Rat No.	Weight on 1-7-38.	18-7-38.	Max. increase.	Food.
9407	42 g.	83 g.	41 g.	Mu 12	9433	51 g.	69 g.	44 g.	Mu 12
9408	43	104	61	12	9434	48	72	24	12
9409	46	102	56	12	9435	48	69	21	12
9410	42	86	44	12	9436	46	69	23	12
9411	44	92	38	12	9437	46	62	16	12
9412	42	84	42	12	9438	50	71	27	12

TABLE VIII.

Rat No	Weight on 13-6-38.	1-7-38.	Max. increase.	Food.
9297	56 g.	77 g.	21 g.	Mu 8
9298	46	58	12	8
9299	46	65	19	8
9300	41	53	12	8
9301	39	51	12	8
9302	46	57	11	8

TABLE IX.

Rat No.	Weight on 9-6-38.	18-7-38	Max. increase.	Food.
9314	57 g.	96 g.	39 g.	Mu 16
9315	48	84	36	16
9316	50	103	53	16
9317	52	82	30	16

DISCUSSION.

In Table II we find that though the vitamins aneurin and lactoflavin were administered in pure forms, they could not neutralise the effects of large amounts of codliver oil when the 3% yeast added was autoclaved. We cannot ascribe the result to the autoclaved yeast alone, since the animals having peanut oil instead of codliver oil had been growing apparently normally though the other factors of the B complex were missing here as with the former groups of animals. In Table III is found that by replacing the 3% autoclaved yeast by dried whole yeast, the animals gained their former weights or more within ten days. After this date the aneurin receiving animals as well as the controls went on gaining weights but all the lactoflavin receiving animals lost weights. In the latter case it is possible that there was some error in the preparation of the food specially there might have been a delay in preparing it fresh within the required time, when the atmosphere being more warm the codliver oil would have turned more toxic. Table IV shows that with the frankonite filtrate of aqueous yeast extract, growth in the animals was appreciably more than with the aneurin administration, specially when we take into consideration the fact that the starting weights in the former case were much below others in our experiments. As a matter of fact in other cases we were not successful with our experiments when so small animals were taken. From Tables V and VI it comes out that extra 12% whole dried yeast is

more effective than frankonite filtrate of yeast extract in neutralising the toxicity of codliver oil and promoting growth. This particular point has all along been noted by De Vries and Puister (*loc. cit.*). Nicotinic acid had all along been found to be ineffective and possibly it is a little toxic as well. Tables VII and VIII show lactoflavin to be more effective than the frankonite filtrate of yeast extract. In Table IX the animals were found to gain weights though autoclaved yeast was used. The difference in this table from Table II is that in Table IX the animals had always a second factor of the B complex in addition to that in Table I, which points to the fact that the different factors of the vitamin B complex together act better.

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NUTRITION STUDIES IN BIHAR. PART II. CHEMICAL COMPOSITION OF SOME LOCAL EDIBLES.

By K. Mitra.

Forty local edibles were analysed chemically as to the moisture, fat, crude fibre, carbohydrate, calcium, phosphorus and iron content. The methods recommended by the American Agriculturists Association were followed during analysis with slight modification. Of the cereal grains *marooa* is the richest source of calcium The various *sattoos* contain fairly high percentage of proteins and minerals Rice and wheat bran contain protective dietary elements in abundance. *Gurh* is rich in calcium.

About a year ago, the intergovernmental conference of the League of Nations (*Report of Intergovernmental Conference*, No. A., 19, 1937, **III**, 69) observed "In various countries a considerable amount of work has been carried out on the nutritive value of foodstuff. The importance of extending and completing investigations of this nature is pointed out". Ranganathan *et al* (*Indian J. Med. Res.*, 1937, **24**, 689) worked out the proximate chemical composition of 200 Indian foods, but out of this comprehensive list only three items were obtained from this province (Darbhanga). During diet survey operations in the province by the author (*Patna J. Med.*, XIV, 1) a need was felt for figures giving the nutritive value of foodstuffs consumed locally. An effort has been made to assess chemically the nutritive value of those local foods which have not so far been analysed at other laboratories in India. The results of investigation of forty items of food are presented in this paper. The methods of analysis were the same as described by Ranganathan *et al* (*loc. cit.*). The list includes 29 kinds of grain foods, 5 kinds of leafy vegetables, 10 kinds of other vegetables, 3 kinds of fish and 2 miscellaneous foods, all purchased from different markets in the city of Patna.

Some of the items of food need explaining. "Besam Boont" is nothing except flour made from dried Bengal gram sold in the market; on the other hand "Boont Sattoo" is the flour made from roasted or baked Bengal gram. Other sattoos (consumed mostly in the rural area) are prepared from the respective pulses or cereals in the same way. "Suthni" is a root vegetable, which is either boiled and eaten as such or dried and flour made out of it for cakes. This food is not popular with the upper classes. "Tilouri" often makes a palatable dish and is prepared by soaking certain pulse grains mostly black gram (or urid), grinding them to a paste and drying them up in small bits.

Table of Food Vatues.

Name of food-stuff.	Botanical name	Moisture.	Protein.	Fat (ether extractives)	Mineral matters.	Carbohydrate.	Crude fibre.	Calcium	Phosphorus.	Iron (in mg.).	Calorific value per 100 g (calculated).	Remarks.
GRAIN FOODS.												
1. Kalamdan rice	*Oryza sativa*	10·47%	6·09%	0·23%	0·56%	82 65%	...	0·003%	0 120%	2·50%	366·0	Raw milled.
2. Ramsal rice	,,	11·00	7·40	0·75	1·12	79·73	...	0·006	0·170	3·00	364 2	Parboiled.
3. Basmati rice	,,	10·01	6·63	0·33	0·71	82·32	...	0·003	0·121	2·55	367·8	Raw milled.
4. Samjira rice	,,	10·33	7·03	0·15	0·74	81·75	...	0·002	0·130	2 73	365·4	,,
5. Whole wheat flour	*Tricitum vulgare*	12·58	12·77	2·40	2·21	68·88	1·16	0 060	0 319	6·32	357·1	
6. Marooa	*Eiousine coracana*	10·88	6·14	1 97	2·05	78 47	0 49	0 301	0·216	4·20	365 2	
7. Water chestnut flour	*Trapa bispinosa*	11·61	10·23	3 22	2·94	72·00	...	0·082	0·424	3 00	367·1	
8. Puffed maize	*Zea mays*	7·35	9·24	4·78	1·02	74·59	2 12	0·005	0·322	37·50	388·6	
9. Puffed paddy (Laoa)	*Oryza sativa*	10·49	8·41	4·74	1·14	69·83	5·39	0 006	0·210	4·62	364·9	
10. Rice bran	,,	14 79	19·06	12·25	16·65	28·31	8 94	0 136	2·844	111·42	308·1	
11. Wheat bran	*Tricitum vulgare*	10·78	13·81	3·95	6·04	55·55	9 87	0·118	1 294	15·00	321·1	
12. Moong dal or green gram	*Phaseolus radiatus*	9·91	24·25	0·91	3·15	61·78	...	0·029	0·373	4 80	361·2	Without outer husk.

Table of Food Values (contd.).

	Name of food-stuff.	Botanical name.	Moisture.	Protein.	Fat (ether extractives).	Mineral matters.	Carbohydrate.	Crude fibre.	Calcium.	Phosphorus.	Iron (in mg.).	Calorific value per 100 g. (calculated).
13.	Besam boont	*Cicer arictinum*	12·20%	23·88%	5·45%	2·34%	55·01%	1·12%	0·044%	0·316%	6·32%	374·1
14.	Boont sattoo	„	14·12	20·38	7·20	2·50	54·54	1·26	0·065	0·318	8·57	374·1
15.	Makai sattoo	*Zea mays*	11·42	9·32	3·55	1·82	71·97	1·92	0·006	0·259	6·32	366·3
16.	Jao sattoo	*Hordeum vulgare*	14·99	12·00	2·37	2·15	67·32	1·17	0·033	0·419	8·00	347·3
17.	Matar sattoo	*Pisum sativum*	11·18	25·43	1·52	2·20	58·61	1·06	0·042	0·295	5·66	358·7
18.	Soojee	*Samolina*	9·28	9·05	0·95	0·56	80·16	...	0·044	0·151	4·00	374·6
19	Sewai or wheat flour fine rolls	*Tricitum vulgare*	10·91	10·94	0·44	0·64	77·07	...	0·029	0·201	2·22	364·9
20.	Kachri or tender Bengal gram	*Cicer arietinum*	83·58	5·53	0·14	0·84	9·91	...	0·056	0·113	1·80	64·6
	LEAFY VEGETABLES											
21.	Pooin sag	*Basseola rubra*	93·14	2·69	0·19	1·26	1·75	0·97	0·086	0·086	27·59	20·0
22.	Lal sag	*Amaranthus blitum*	88·40	2·94	0·20	2·02	5·59	0·85	0·120	0·073	18·18	36·8
23.	Palki sag	*Spinacea oleracia*	91·59	1·80	0·21	1·05	4·53	0·82	0·212	0·082	22·34	27·9
24.	Boont sag	*Cicer arictinum*	87·97	2·76	0·81	2·46	5·35	0·65	0·272	0·160	12·69	40·8
25.	Khesari sag	*Lathyrus sativa*	89·00	3·29	0·65	1·10	5·24	0·72	0·310	0·108	4·36	41·0

Table of Food Values (contd.).

	Name of food-stuff.	Botanical name.	Moisture.	Protein.	Fat (ether extractives).	Mineral matter.	Carbohydrate.	Crude fibre.	Calcium.	Phosphorus.	Iron (in mg.).	Calorific value per 100 g. (calculated).	Remarks.
	Other Vegetables												
26.	Parwar or Patol	*Trichosanthes dioica*	91·00%	1·73%	0·39%	0·64%	4·13%	2·11%	0·026%	0·080%	0·58%	27·7	
27.	Kundri (Telakucho)	*Cephalandra indica*	96·23	1·78	0·15	0·43	0·78	0·63	0·020	0·047	0·38	11·9	
28.	Kheksa or Chathail Kankrol	*Momordica chochinchinensis*	84·09	2·61	0·66	1·02	5·69	5·93	0·021	0·148	2·59	40·2	
29.	Kadam	*Anthecephalus cadamba*	82·11	1·42	0·12	0·96	15·39	...	0·081	0·067	1·61	70·0	
30.	Suthni		75·89	3·94	0·23	1·92	18·13	0·79	0·021	0·094	0·75	92·6	With skin.
31.	Arui	*Colocasia antiquorum*	75·24	2·55	0·10	1·12	20·32	0·67	0·030	0·091	0·79	94·7	
32.	Nenua	*Luffa aegyptica*	93·66	1·41	0·07	0·40	3·58	0·88	0·018	0.056	0·49	21·1	
33.	Barsema (Makhan seem).	*Canavalia gladiata*	84·21	2·20	0·22	0·76	11·59	1·02	0·086	0·057	0·73	58·6	
34.	Seem or Broad bean	*Dolichos lablab*	86·43	2·90	0·24	0·88	7·57	1·98	0·091	0·089	1·20	45·2	
35.	Kankri	*Cucumis melo*	93·73	0·84	0·10	0·50	4·10	0·73	0·028	0·049	0·94	21·2	
	Fish												
36.	Rehu Fish	*Labeo rohita*	78·09	17·82	2·77	1·32	...	...	0·090	0·277	0·62	98·8	
37.	Bachwa Fish	*Clupisoma garua*	76·37	18·13	4·40	1·10	...	...	0·062	0·223	0·53	115·2	
38.	Bhoona Fish (chittal)		77·30	17·56	3·70	1·44	...	...	0·082	0·281	0·72	97·1	
	Miscellaneous												
39.	Gurh		8·86	0·25	0·05	1·00	89·84	...	0·400	0·045	7·06	369·8	
40.	Tilouri		12·23	21·81	0·96	3·12	61·88	...	0·174	0·389	2·35	352·1	

Discussion.

Of the four varieties of local rice analysed the mineral content (as also calcium and phosphorus) was found comparatively high in Ramsal, which is nothing other than parboiled Basmati grains. Amongst the cereal grains "Marooa" was found to be richer in the most important mineral element calcium. Unfortunately this food grain is associated with social taboo and as such is rarely used by the middle classes. Only very poor people, mostly of the lower castes, make flour out of this grain for unleavened cakes and cousume them as such. If only the people in the lower middle classes could be persuaded to use marooa flour for milk porridge, the same would serve as a cheap and nutritive breakfast for children. Puffed paddy is also comparatively rich in mineral salts.

Except the *makai*, other sattoas have got very high protein and mineral content, the calcium yield is also fairly moderate; thus sattoos form a highly nutritive, easily portable and ready-made group of edibles. These powdered grain foods need only water to make them into a dough, which with a pinch of salt and green chillies makes an excellent and wholesome repast on occasions when cooking becomes inconvenient or impossible.

All the five types of leafy vegetables examined show fairly high calcium values. The first three in the list are consumed only after cooking, but the last two ("Boont and Khesari sāg") are consumed in a raw state when the plants are still very tender. With the growth of urbanisation this healthy practice is becoming unpopular, if not obsolete. "Gurh" was found to contain an unusually high percentage of calcium, this was evidently due to the fact that during the process of manufacture, the thickened and highly coloured juice of sugar cane is treated with lime for clarification. Calcium of the lime is retained in Gurh to a very large extent. From the point of view of nutrition the superiority of Gurh over sugar can hardly be overrated.

Rice and wheat brans (polishings) are used exclusively as cattle fodder, though they contain all the proximate principles of food and mineral elements in very large proportions. The protein content of the rice bran was found to be particularly high (19·06 %) and it stands to reason that if these polishings be utilised for the preparation of some edibles for the children, sufficient protective food would be available at a very low cost. Basu and Basak (*Indian J. Med. Res.*, 1937, **24**, 1043) have found that at 5% level of protein diets rice polishings from the Bengal variety of grains failed to produce growth on young rats, but maintenance was usually observed. On

the other hand Wan (*Chinese J. Physiol.*, 1935, **9**, 140) made an attempt to process wheat bran for human consumption by soaking, steaming, frying and fermenting, and found that wheat bran could be utilised to the extent of 15 % in place of more refined cereal in an ordinary vegetarian diet. In a poor country like India, the possibilities of the utilisation of rice and wheat brans with their high nutritive values for hnman consumption, deserves more than a passing notice.

Regarding the iron content, at the present state of our knowledge. nothing need be said except that some of the unusually high figures are evidently due to handling those stuff with iron implements and utensils.

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THE REACTION OF SOME AROMATIC DIAMINES WITH ETHYL MALONATE.

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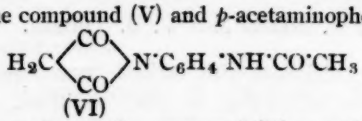
The reaction of ethyl malonate with various monoamines gives malonamides and malonamates according to the conditions of the reaction (Whitely, *J. Chem. Soc.*, 1903, **83**, 24). Since in the case of diamines the number of products expected will be greater, a study of the reaction of malonic ester with benzidine, tolidine and *p*-phenylenediamine has been undertaken varying (*i*) the concentrations of the ester and base, (*ii*) the temperature of the reaction and, (*iii*) the medium of reaction.

By reacting malonic and succinic acids with *o*- and *p*-phenylenediamines, Meyer (*Annalen*, 1903, **327**, 1) isolated various products, *e.g.*, *o*-phenylenesuccinamide, N : N′-di-*o*-aminophenylsuccinamide, *o*-phenylene-amidinesuccinic acid and *p*-phenylene-disuccinimide. Later, the same author (*Annalen*, 1906, **347**, 17) prepared diethyl *p*-phenylenedimalonamate by reacting *p*-phenylenediamine with malonic ester. Malonylbenzidine, $CH_2(CO{\cdot}NH{\cdot}C_6H_4)_2$, was obtained by Ramfrey (*J. Chem. Soc.*, 1911, **99**, 621) by heating malonic ester with benzidine at 210°.

The reaction between equimolecular proportions of ethyl malonate and benzidine at 140-60° yielded two substances melting above 300°. On increasing the proportion of ester and lowering the reaction temperature to 115-120°, the reaction product was found to consist of ethyl 4-aminodiphenyl-4′-malonamate $H_2N{\cdot}C_6H_4{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$ (I), diethyl diphenylene-4 : 4′-dimalonamate $(\cdot C_6H_4{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et)_2$ (II), and malonylbenzidine (III). The presence of the amino group in compound (I) was proved by diazotising it and coupling with β-naphthol. The compound (II), containing free ester groups, underwent hydrolysis to the corresponding acid which on decarboxylation gave diacetylbenzidine. The compound (I), on heating at 140° for about 4 hours, gave the compounds (II), (III) and benzidines while on boiling with xylene for about 10 hours passed exclusively into (III). The results of comparative yields from the experiments carried out with different proportions of the reacting substances at different temperatures are given in Table I (*vide* experimental).

In the case of tolidine only two compounds could be isolated corresponding to types (II) and (III) from benzidine, although this reaction was carried out at lower temperatures and with excess of ester.

The reaction of malonic ester with *p*-phenylenediamine gave (*i*) diethyl *p*-phenylene-dimalonamate (IV), (*ii*) ethyl *p*-acetaminophenylene-malonamate (V) and an insoluble blue mass. The same reactants after 12 hours' boiling in xylene gave the compounds (IV) and (V), while if the heating was continued for 24 hours, the resulting mass gave only (V) and an insoluble residue. The compound (IV) on heating at 160° for about 4 hours gave the compound (V) and *p*-acetaminophenylenemalonimide (VI),



(VI)

while in boiling xylene only compound (V) was obtained. The compounds (IV) and (V), which on heating at 180-85° also passed into compound (VI), on hydrolysis gave their respective acids which on decarboxylation furnished diacetyl-*p*-phenylenediamine.

EXPERIMENTAL.

Reaction of Ethyl Malonate with Benzidine.—A mixture of ethyl malonate (0·3 mol.) and benzidine (0·1 mol.) was heated in an oil-bath at 115-20° for 8 hours allowing the alcohol formed to distil over. The reaction product after cooling was filtered, washed with alcohol to remove the unreacted ester and amine, and then repeatedly extracted with boiling alcohol and filtered hot leaving a residue (A). The filtrate on cooling deposited shining plates of diethyl diphenylene-4:4′-dimalonamate (II). The filtrates from (II) on concentration and dilution with equal amount of water yielded *ethyl 4-aminodiphenyl-4′-malonamate* (I), which is very soluble in cold alcohol, sparingly soluble in benzene and practically insoluble in petroleum, ether and water, m. p. 139-40°. (Found : C, 68·2 ; H, 6·2 ; N, 9·2. $C_{17}H_{18}O_3N_2$ requires C, 68·4; H, 6·0; N, 9·4 per cent).

Azo Derivative.—Ethyl 4-aminodiphenyl-4′-malonamate was diazotised and coupled with β-naphthol as usual. The colouring matter on recrystallisation from alcohol was obtained as bright red shining plates, m.p. 298° (decomp.). (Found: N, 9·2. $C_{27}H_{23}O_4N_3$ requires N, 9·2 per cent).

Ethyl 4-aminodiphenyl-4′-malonamate was heated in an oil-bath at 140° for about 4 hours. The resulting mass on extraction with boiling alcohol gave (*i*) a residue insoluble in alcohol, (*ii*) diethyl diphenylene-4:4′-dimalonamate (II) (*vide infra*). (Found : N, 7·0 per cent) and, (*iii*) some benzidine.

Diethyl diphenylene-4:4′-dimalonamate (II) is sparingly soluble in hot alcohol and practically insoluble in cold alcohol, benzene, petroleum, ether, etc, m.p. above 300°. (Found: C, 64·1; H, 5·9 ; N, 6·8. $C_{22}H_{24}O_6N_2$ requires C, 64·0 ; H, 5·8; N, 6·8 per cent).

Hydrolysis of Diethyl diphenylene-4:4′-dimalonamate (II).—The above mentioned compound was refluxed on the water-bath with the calculated amount of alcoholic sodium hydroxide. When the reaction was complete (about 1 hour) the solution was filtered hot and acidified when the free acid separated out. It was filtered and crystallised from glacial acetic acid in which it was very sparingly soluble, m.p. 323-24° (decomp.). It is practically insoluble in water, alcohol, benzene, petroleum and ether. The sodium salt of the acid was crystallised from water. (Found : N, 6·7 ; Na, 11·5. $C_{18}H_{14}O_6N_2Na_2$ requires N, 7·0 ; Na, 11·5 per cent). The free acid was decarboxylated at 300° to diacetylbenzidine, m.p. 317°.

Malonylbenzidine (III).—The residue (A) is practically insoluble in all ordinary organic solvents except in boiling nitrobenzene in which it is very sparingly soluble, m.p. above 300°. (Found : C, 71·6 ; H, 5·0; N, 10·8. $C_{15}H_{12}O_2N_2$ requires C, 71·4 ; H, 4·8; N, 11·1 per cent).

Ethyl 4-aminodiphenyl-4′-malonamate (I) was also heated in boiling xylene for about 10 hours. The resulting mass was filtered, washed with petroleum to remove xylene, dried and then repeatedly extracted with boiling alcohol. Nothing separated from alcoholic filtrates. The insoluble residue was identified to be malonylbenzidine. (Found : N,11·3. $C_{15}H_{12}O_2N_2$ requires N, 11·1 per cent).

TABLE I.

Comparative Yields of Compounds I, II *and* III *from Experiments carried out with different Proportions of Ethyl malonate and Benzidine at different Temperatures.*

Benzidine.	Malonic ester.	Temp.	Yields of compounds I.	II.	III.
18·4 g. (0·1 mol.)	32·0 g. (0·2 mol.)	120-25°	2 g.	7 g.	17 g.
18·4 (0·1 mol.)	48·0 (0·3 mol.)	120-25°	6·5	9·1	9·1
18·4 (0·1 mol.)	48·0 (0·3 mol.)	115-120°	8·4	5·5	3·3

Reaction of Ethyl Malonate with Tolidine.

A mixture of ethyl malonate (0·3 mols) and tolidine (0·1 mol.) was heated as in the first experiment at 120° for about 6 hours. The reaction product was then filtered, washed with little alcohol to remove unreacted base and ester and then extracted several times with boiling alcohol. The filtrates on cooling deposited diethyl di-*o*-tolyl-4:4′-dimalonamate. Nothing separated from filtrates either on dilution with water or on evaporation, thus indicating the absence of the compound of type (I). The residue left after

extraction with boiling alcohol was practically insoluble in the usual organic solvents. *Diethyl di-o-tolyl-4:4'-dimalonamate* is sparingly soluble in boiling alcohol, practically insoluble in cold alcohol, benzene, petroleum, ether, m. p. above 300°. (Found: C, 65·6 ; H, 6·7 ; N, 6·7. $C_{24}H_{28}O_6N_2$ requires C, 65·4 ; H, 6·4 ; N, 7·4 per cent).

Reaction of Ethyl Malonate with p-*Phenylenediamine*.—A mixture of ethyl malonate (0·3 mol.) and *p*-phenylenediamine (0·1 mol.) was heated at 115-120° for about 10 hours and the product on working up as usual yielded (*i*) ethyl *p*-acetaminophenylmalonamate (V). (*ii*) ethyl *p*-phenylene-dimalonamate (IV), and (*iii*) a blue powder practically insoluble in usual organic solvents (Found : N, 13·9 per cent). On carrying out the reaction in presence of xylene at 140-50° for 12 hours, the compounds (IV) and (V) with traces of (VI) were obtained; on prolonging the heating to 24 hours only the compound (V) with traces of (VI) could be obtained.

Hydrolysis of Diethyl p-*phenylenedimalonamate* (IV).—Ethyl *p*-phenylene-dimalonamate (IV) on hydrolysis with the calculated amount (1·2 mol.) of alcoholic sodium hydroxide yielded the corresponding acid which when heated began to turn black above 250°, shrink at 280° and melt with decomposition at about 300°. (Found: N, 9·9 ; M.W., 280·3. $C_{12}H_{12}O_6N_2$ requires N, 10·0 per cent. M.W., 280·0). This acid on decarboxylation gave diacetyl *p*-phenylenediamine, m.p. 204°.

Action of Heat on Diethyl p-*Phenylenedimalonamate*.—Ethyl *p*-phenylene-dimalonamate was heated in an oil-bath at 160° for about 4 hours and the resulting mass with an ester-like odour on working up as usual gave (*i*) a residue insoluble in alcohol and other organic solvents (Found : C, 60·8; H, 4·7 ; N, 12·8. $C_{11}H_{10}O_3N_2$ requires C, 60·6; H, 4·6 ; N, 12·9 per cent). (*ii*) *p*-acetaminophenylmalonimide (VI), and (*iii*) ethyl *p*-acetaminophenyl malonamate (Found : C, 59·1 ; H, 6·0 ; N, 10·6 per cent).

Ethyl p-*acetaminophenyl-malonamate* (V) is sparingly soluble in boiling alcohol and practically insoluble in cold alcohol, benzene, petroleum and ether, m.p. above 300°. (Found: C, 59·0; H, 6·0; N, 10·5. $C_{13}H_{16}O_4N_2$ requires C, 59·1 ; H, 6·0 ; N, 10·6 per cent). On hydrolysis with the calculated amount of alcoholic sodium hydroxide as usual, the corresponding acid, practically insoluble in the usual organic solvents, was obtained, m.p. about 300° (decomp.) with shrinking at 280°. (Found: N, 11·7. $C_{11}H_{12}O_4N_2$ requires N, 11·9 per cent).

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COMPLEX COMPOUNDS OF BIGUANIDE WITH TERVALENT METALS. PART V. THIOCYANATES OF CHROMIUM BIGUANIDES.

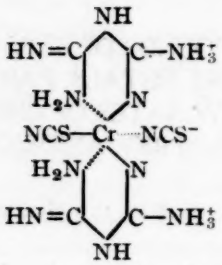
By Priyadaranjan Rây and Haribola Saha.

A new type of chromium *bis*biguanide complex—diacido chromium *bis*biguanide thiocyanate and nitrate—has been prepared and their properties studied. In addition, preparation and properties of chromium *tris*biguanide hydroxo-thiocyanate, trithiocyanate and hydroxo-aquo-chromium *bis*biguanide thiocyanate have been described.

In previous communications of this series various salts of chromium *bis*biguanide and hydroxo-aquo-chromium *bis*biguanide (Rây and [illegible], *Indian Chem. Soc.*, 1937, **14**, 670 ; 1938, **15**, 353 ; Rây and Ghosh, [illegible] 15, 347, 350) have been described, excluding the thiocyanates. Attempts to prepare these latter have led to certain interesting results. When an aqueous solution of chromium *tris*biguanide hydrate is treated with a solution of ammonium thiocyanate, the crystalline precipitate, which separates from the liquid, is a *tris*biguanide hydroxo-thiocyanate with strongly alkaline character. From the filtrate neutral bright red crystals of a substance containing two biguanide molecules and three thiocyanate radicals per chromium atom separate after some time. The only possible constitution for a compound of this composition, in keeping with the hexa co-ordination of chromic chromium, is given by

$$\left[(\mathrm{BigH^+})_2 \cdot \mathrm{Cr} \begin{matrix} \cdot(\mathrm{SCN}) \\ \cdot(\mathrm{NCS}) \end{matrix}^- \right] \mathrm{SCN},$$

in which the central chromium atom has two of its primary valencies satisfied by combination with two biguanide molecules as usual, while its third primary valency binds one of the thiocyanate radicals inside the complex zone. The other thiocyanate radical in the same zone is really a negative ion, with its positive counterpart represented by a $-\mathrm{NH}_3^+$ group of one of the biguanide molecules, and is linked co-ordinatively to the central chromium atom through nitrogen. This is illustrated by the following figure :



The substance, however, gradually hydrolyses in aqueous solution into aquo-compounds to form mono- and diaquo-chromium *bis*biguanide thiocyanate as indicated by the cryoscopic and conductivity measurements. Silver nitrate precipitates practically the whole of the thiocyanate from the solution at the room temperature. At lower temperatures (10-15°) a part of the thiocyanate, however, remains in the solution. Cryoscopic measurement for the molecular weight gives a value (170) which is lower than half (214) but greater than one-third (142·6) of the formula weight. Conductivity measurements at 15°, however, furnishes evidence of considerable hydrolysis of the diaquo-derivative. The aqueous solution of the is also slightly acid to litmus as could be expected from the formation of an aquo-salt.

Attempts to prepare the corresponding nitrate by double decomposition with silver nitrate led only to the diaquo-nitrate—an evidence for the hydrolytic dissociation of the thiocyanate. But this diaquo-nitrate is salt converted into dinitrato-chromium *bis*biguanide nitrate by losing water at 125°.

The hydrolysis of the salt can, therefore, be represented as :

$$[(BigH^+)_2\cdot Cr\cdot{}^{\cdot SCN}_{\cdot NCS^-}]\,SCN + H_2O \longrightarrow [(BigH^+)_2\cdot Cr\cdot{}^{\cdot SCN}_{\cdot OH_2}](SCN)_2$$

$$\downarrow H_2O$$

$$[(BigH^+)_2\cdot Cr\cdot{}^{\cdot OH_2}_{\cdot OH_2}](SCN)_3$$

In addition to this chromium *bis*biguanide thiocyanate, which represents a new type (diacido) of *bis*biguanide series, chromium *tris*biguanide trithiocyanate and hydroxo-thiocyanate, as well as hydroxo-aquo-chromium *bis*biguanide thiocyanate have been described in this paper.

EXPERIMENTAL.

Chromium tris*Biguanide Hydroxo-thiocyanate.*—When a strong ice-cold solution of chromium *tris*biguanide hydroxide was treated with a strong solution of ammonium thiocyanate in excess, a dull red crystalline precipitate separated gradually on stirring. This was filtered off, as it was found to be a mixture of two or more products, possibly of chromium *tris*biguanide dihydroxo-monothiocyanate and monohydroxo-dithiocyanate, which could not be separated by recrystallisation. The filtrate from the above precipitate was allowed to stand overnight at the room temperature, when a second crop of purer monohydroxo-dithiocyanate was obtained in the form of duli red crystals. These were filtered, washed first with a little ice-cold water and then with alcohol. The crystals were afterwards dried in an atmosphere free from CO_2 over moist NaOH,

The reaction occurs probably as follows.

$$[Cr.(BigH^+)_3](OH)_3 + NH_4SCN \longrightarrow [Cr.(BigH^+)_3]\begin{matrix}(OH)_2\\SCN\end{matrix} + NH_3 + H_2O$$

$$\Big\downarrow NH_4SCN$$

$$[Cr.(BigH^+)_3]\begin{matrix}(OH)\\(SCN)_2\end{matrix} + NH_3 + H_2O$$

{Found: N, 47·25 ; S, 12·70 ; Cr, 10·50. $[Cr,(BigH^+_3)]\begin{matrix}(OH)\\(SCN)_2\end{matrix}\cdot H_2O$ requires N, 47·0 ; S, 12·60 ; Cr, 10·30 per cent}.

The substance forms dull red crystals, moderately soluble in water and strongly alkaline to litmus. It liberates ammonia from ammonium salts on warming.

Chromium bis*Biguanide Trithiocyanate.*—This new type of compound was obtained from the filtrate of the above described hydroxo-thiocyanate on keeping for a few days. The same substance was also obtained directly by heating on the water-bath a mixture of concentrated solutions of chromium *tris*biguanide hydroxide and ammonium thiocyanate till all free ammonia escaped from the solution. The latter was then kept in a desiccator over concentrated H_2SO_4 when the *bis*biguanide trithiocyanate separated in beautiful red crystals. These were washed first with little ice-cold water and then with alcohol. The crystals, thus obtained, were quite pure. In some preparations they were recrystallised from a little water. These were dried in air.

The reaction may be represented as follows:

$$[Cr.(BigH^+)_3](OH)_3 + NH_4SCN = [(BigH^+)_2 Cr.{}^{SCN}_{NCS^-}]SCN + BigH.HSCN + 4NH_3 + 3H_2O.$$

{Found: N, 42·85, 43·1 ; S, 22·3, 22·2 ; Cr, 12·3, 12·35.

$[BigH^+)_2 Cr.{}^{SCN}_{NCS^-}]$ SCN requires N, 42·50 ; S, 22·40; Cr, 12·15 per cent}.

The substance is readily soluble in water and reacts very slightly acid to litmus. From a cold concentrated solution, the whole of the thiocyanate is not immediately precipitated by silver nitrate. Hydrolysis, however, occurs readily in aqueous solution on dilution or warming, as indicated by the cryoscopic and conductivity measurements.

Cryoscopic measurement.

Substance, (g/100 c.c.)	Depression. Δ	Mol. wt. m	Vant Hoff's factor $i=M/m$.
0·7418	0·078	171·2	2·5
0·3709	0·040	167·0	2·56

M = mol. wt. calculated = 428.

The result seems to suggest a partial hydrolysis into:

$$[(BigH^+)_2 Cr.{}^{SCN}_{OH_2}](SCN)_2.$$

Molecular conductivity.

($t=18°$).

v (dilution)	32	64	128	256	512	1024
μ_v ...	188·0	216·2	230·0	248·2	271·5	276·6

Molecular conductivity at 25° for μ_{1000} of $[Cr\{CO(NH_2)_2\}_6](SCN)_3$ and $[(NH_3)_4 Cr.{}^{OH_2}_{Cl}](NO_3)_2$ are respectively 325·5 and 260·3 (Werner and Kalkmann, *Annalen*, 1902, **322**, 313 ; Werner and Miolati, *Z. physikal. Chem.*, 1894, **14**, 515).

Taking note of the difference in temperatures, it may, therefore, be concluded that the substance hydrolyses more or less completely at 18° into the diaquo-derivative $[(BigH^+)_2.Cr.(H_2O)_2](SCN)_3$.

Chromium bis*Biguanide Trinitrate and its Diaquo-derivative.*

Finely powdered chromium *bis*biguanide trithiocyanate (2·5 g.) was made into a paste with water and the solution of a calculated quantity (3 g.) of silver nitrate in a little water was added, drop by drop, to the paste,

agitating or shaking the mixture all the time. The precipitate of silver thiocyanate was filtered off. The filtrate was kept overnight in a vacuum desiccator over H_2SO_4. The concentrated solution, when cooled in ice, deposited crystals of brick-red colour. These were filtered, washed first with a little ice-cold water, then with alcohol and finally dried in air. {Found : N, 36·90; Cr, 10·75. $[(BigH^+)_2 \cdot Cr \cdot (H_2O)_2](NO_3)_3,H_2O$ requires N, 36·84 ; Cr, 10·52 per cent}.

Thus the fully hydrolysed or diaquo-derivative separates in the case of the nitrate. But the substance lost practically the whole of the water on heating to 120-125°. The loss of weight corresponded to 10·92%.

Calculated from the formula, H_2O = 10·93%. The anhydrous compound gave on analysis N, 41·09.

$$[(BigH^+)_2 \cdot Cr \cdot \begin{matrix} NO_3 \\ NO_3 \end{matrix}^-]NO_3 \text{ requires N, 41·36 per cent.}$$

This shows that the hydrated diaquo-derivative, on losing water, is transformed into the chromium *bis*biguanide trinitrate of the corresponding thiocyanate type, from which the diaquo-nitrate was obtained by double decomposition.

The anhydrous chromium *bis*biguanide trinitrate, also brick-red in colour, very slowly absorbs water on exposure to air, changing gradually into the original diaquo-derivative after a long time.

Chromium tris*Biguanide Trithiocyanate.*

This normal salt was prepared by double decomposition from chromium *tris*biguanide sulphate and barium thiocyanate. The finely powdered complex was agitated in a mortar with a concentrated solution of a calculated amount of barium thiocyanate. The solution, after the reaction was complete, must not contain any barium or sulphate ion. The filtrate from the barium sulphate was cooled in ice when dark red crystals of the normal thiocyanate separated from the solution. These were washed first with ice-cold water and then with alcohol. A second crop was obtained from the mother-liquor by evaporating over H_2SO_4 in vacuum.

The air-dried sample was analysed. {Found: N, 47·80; S, 18·28; Cr, 9·97. $[Cr(BigH^+)_3](SCN)_3$ requires N, 47·63; S, 18·15; Cr, 9·83 per cent}.

The substance forms bright red crystals, readily soluble in water. Cryoscopic measurement of aqueous solution indicates partial hydrolysis into *bis*biguanide derivative. This accounts for the fact that the normal *tris*biguanide trithiocyanate could not be obtained by the action of ammonium thiocyanate on *tris*biguanide hydroxide.

Cryoscopic measurement.

Substance. (g/100 c.c.)	Depression. Δ	M. W. *m*.	Vant Hoff's factor $i = M/m$.
0·5355	0·081	119	4·45
0·26775	0·042	115	4·60

M(Calc. mol. wt.) = 529.

Hydroxo-aquo-chromium bis*Biguanide Thiocyanate.*

The filtrate from the chromium *tris*biguanide thiocyanate was allowed to remain at the ordinary temperature for some days, when the colour of the solution gradually changed from dark red to red-violet. This, on concentration in vacuum over H_2SO_4 and cooling in ice, gave red-violet crystals of the hydroxo-aquo complex. The crystals were washed first with a little ice-cold water and then with alcohol. For the purpose of analysis they were dried in air freed from CO_2. {Found: N, 41·40; S, 15·80; Cr, 13·0. $\left[(BigH^{+})_2.Cr.\begin{matrix}.OH\\.OH_2\end{matrix}\right](SCN)_2$ requires: N, 41·48; S, 15·80; Cr, 12·84 per cent}.

The substance is readily soluble in water forming a slightly alkaline solution like all hydroxo-aquo compounds previously described (*loc. cit.*).

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SPIRO-COMPOUNDS. PART V. THE FORMATION AND TRANSFORMATION OF SPIRO-COMPOUNDS FROM 3-AND 2-METHYL*CYCLO*HEXANONES.

By Nripendra Nath Chatterjee and Girindra Nath Barpujari.

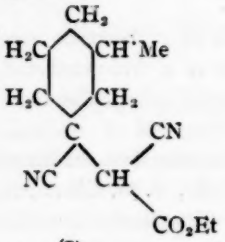
Spiro-compounds have been built up from methyl*cyclo*hexanone to examine, on the basis of the valency deflexion hypothesis, the nature of strain in *cyclo*hexane ring in which *ortho* or *meta* positions are occupied by groups. β-Methylnaphthalene has been obtained as a product of dehydrogenation of 3-methyl*cyclo*hexane-spiro-*cyclo*pentane-5′-carboxylic acid.

*cyclo*Hexane, which behaves as a strained ring, becomes strainless when interlocked in the *ortho* position with a six- or a five-membered ring (*cf.* Rao, *J. Chem. Soc.*, 1929, 1954; *ibid.*, 1930, 1162; Kandiah, *ibid.*, 1931, 952). This behaviour and isomerism arising out of *cis* and *trans* lockings of two monocyclic rings have been explained on the assumption of their existence in multiplanar forms (*cf.* Mohr, *J. pr. Chem.*, 1918, *ii*, **98**, 315; *Ber.*, 1922, **55**, 230; Hückel and collaborators, *Nachr. Gess. Wiss. Göttingen*, 1923, 43; *Annalen*, 1924, **441**, 1; 1926, **451**, 109; *Ber.*, 1925, **58**, 447, 1449; 1932, **65**, 2137; Hückel and Friedrich, *Annalen*, 1926, **451**, 132; Kandiah, *loc. cit.*). As it has not been possible yet to isolate the two strainless forms of *cyclo*hexane or its monosubstituted derivatives (Werner and Conard, *Ber.*, 1899, **32**, 3046; Wightman, *J. Chem. Soc.*, 1926, 2541) the postulate of Mohr (*loc. cit.*) that they are easily interconvertible holds good. From physical considerations based on the energy associated with angular strain in homocyclic ring it might be anticipated that *cyclo*hexane ring should change from one form to the other at almost every collision. Such conversion being less expected in the di-derivatives, it has been possible to separate the strainless isomers (Qudrat-i-Khuda, *J. Indian Chem. Soc.*, 1931, **8**, 277,442; *Nature*, 1935, **136**, 301; Goldschmidt and Grafinger, *Ber.*, 1935, **68**, 279; Dey and Linstead, *J. Chem. Soc.*, 1935, 1063; Desai, Hunter, Khan and Sahariya, *ibid.*, 1936, 416). It appeared of interest to examine on the basis of the valency deflexion hypothesis of Thorpe and Ingold (*J. Chem. Soc.*, 1915, **107**, 1080) the nature of strain in the *cyclo*hexane ring with *ortho* or *meta* positions substituted by groups.

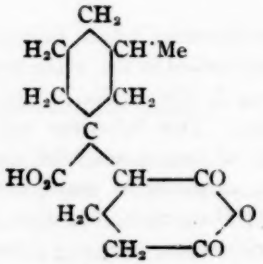
3-Methyl*cyclo*hxanone cyanohydrin reacts with the sodium salt of ethyl cyanoacetate to yield the sodium salt of ethyl 1-cyano-3-methyl-

*cyclo*hexane-1-cyanoacetate (I), which with ethyl β-chloropropionate gives diethyl 1-cyano-3-methyl*cyclo*hexane-1-α-cyanoglutarate. On hydrolysis, the above ester yields the anhydride (II) (corresponding ester is obtained on esterification) from which the required 1-carboxy-3-methyl*cyclo*hexane--1-α-glutaric acid is obtained after treatment with alkali. The ester (III), obtained from the above acid, yields diethyl 3-methyl*cyclo*hexane-spiro-*cyclo*pentane-2′-one-3′:5′-dicarboxylate (IV) on treatment with sodium. It is hydrolysed by means of dilute sulphuric acid (20%) to yield 3-methyl-*cyclo*hexane-spiro-*cyclo*pentane-2′-one-5′-carboxylic acid (V), also obtained by effecting the ring closure according to the method of Perkin and Thorpe (*J. Chem. Soc.*, 1904, **85**, 128).

Similar compounds have been obtained from 2-methyl*cyclo*hexanone-cyanohydrin.



(I)



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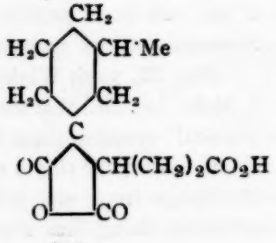

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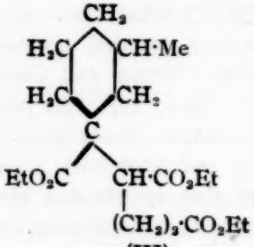

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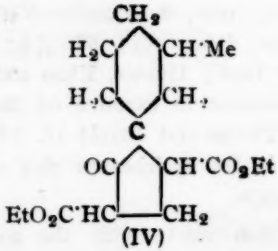

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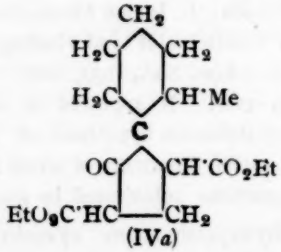
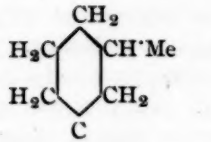


(IV*a*)



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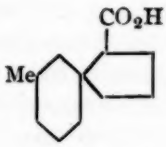

(VI)

The spiro-compounds (IV) (IV*a*) differ in no way from the *cyclo*hexane analogue with respect to their formation (Chatterjee, *J. Indian Chem. Soc.*, 1936, **13**, 536). As no suitable reagent could be found which attacks the one, leaving the other intact, nothing can be said at present as regards the difference in their stability. Isolation of the strainless isomers of compounds in which the damping effect of the spiro-*cyclo*pentane ring is expected, is now being tried.

Incidentally it might be mentioned that our knowledge of the ring transformation during selenium dehydrogenation is limited. An observation in this line has been made in which β-methylnaphthalene has been produced from 3-methyl*cyclo*hexane-spiro-*cyclo*pentane-5′-carboxylic acid (VI) obtained by the Clemmensen reduction of the keto acid (V).

Experiments are in progress to study the ring transformation of the spiro-compounds from 2-methyl*cyclo*hexanone.

EXPERIMENTAL.

Diethyl 1-Cyano-3-methylcyclohexane-1-α-cyanoglutarate. — 3-Methyl-*cyclo*hexanone cyanohydrin was prepared in good yield as follows: 3-Methyl*cyclo*hexanone (100 g.) was shaken with a solution of sodium bisulphite (200 g.) in water (250 c.c.) and the ice-cold mixture gradually treated with a solution of potassium cyanide (80 g.) in water (150 c.c.). After 2 hours the cyanohydrin was extracted by means of ether, washed with water, and then with a saturated solution of sodium chloride, dried (anhydrous sodium sulphate), and the solvent removed with the addition of two drops of concentrated sulphuric acid. It was then distilled under reduced pressure, b.p. 132-135°/20 mm. To a well cooled solution of this freshly distilled 1-hydroxy-1-cyano-3-methyl*cyclo*hexane (134 g.)

in absolute alcohol (134 c.c.), a suspension of ethyl sodiocyanoacetate, obtained from ethyl cyanoacetate (118 g.), sodium (23·3 g.) and alcohol (354 c.c.), was gradually added with vigorous shaking. The mixture after being kept at 0° for 6 hours and at room temperature for 3 days, was mixed with ethyl β-chloropropionate (160 g.) and after the initial reaction had abated, boiled under reflux until a test portion, diluted with water, was neutral to litmus (about 40 hours). The mixture was filtered, washed with dry ether and the filtrate diluted with water and extracted with ether; the ethereal extract was washed with a large volume of water to remove alcohol, dried and ether recovered. It distilled as a viscous liquid, b.p. 216°/5 mm., yield 100 g. (Found: C, 64·5; H, 7·5. $C_{18}H_{26}O_4N_2$ requires C, 64·6; H, 7·7 per cent).

Diethyl 1-Cyano-2-methylcyclohexane-1-α-cyanoglutarate.—It was obtained from freshly distilled 1-hydroxy-1-cyano-2-methyl*cyclo*hexane as described above. In a typical experiment the cyanohydrin (96 g.) in absolute alcohol (96 c.c.) was treated with the sodium salt of ethyl cyanoacetate (84 g.) and ethyl β-chloropropionate (119 g.). The ester distilled as a viscous liquid, b.p. 212°/5 mm.. and solidified on standing in a vacuum desiccator over sulphuric acid and crystallised from ethyl alcohol in colourless crystals, m.p. 61°, yield 55 g. (Found: N, 8·5. $C_{18}H_{26}O_4N_2$ requires N, 8·38 per cent).

1-Carboxy-3-methylcyclohexane-1-α-glutaric Acid.—The cyano ester (100 g.) was mixed with 6 volumes of 70% sulphuric acid and boiled under reflux for 12 hours. The condenser was removed from the flask from time to time to allow the alcohol formed to escape. The solution was then diluted with water and extracted with ether and the acid thus obtained, dissolved in sodium carbonate solution. The solution was diluted, acidified and extracted with ether. After removing ether, the product was kept in a desiccator where it solidified. It was crystallised from ether, m.p. 185° (decomp.), yield 50 g. (Found: C, 57·4; H, 6·9; Equiv., 91·8. $C_{13}H_{20}O_6$ requires C, 57·3; H, 7·3 per cent. Equiv., 90·67).

The crude anhydride (II) was esterified by passing alcohol vapour through a mixture of it in alcohol containing sulphuric acid. The anhydride ester obtained after working up in the usual manner was distilled in vacuum, b.p. 187°/4 mm. (Found: C, 63·73; H, 7·83. $C_{15}H_{22}O_5$ requires C, 63·8; H, 7·8 per cent).

1-Carboxy-2-methylcyclohexane-1-α-glutaric acid was obtained by the hydrolysis of diethyl 1-cyano-2-methyl*cyclo*hexane-1-α-cyanoglutarate as described above, m.p. 170° (decomp.), yield 25 g. from 55 g. of the cyano ester. (Found: C, 57·68; H, 6·86. $C_{13}H_{20}O_6$ requires C, 57·3; H, 7·3 per cent).

The anhydride ester boiled at 185-190°/4 mm. (Found: C, 63·9; H, 8·2. $C_{15}H_{22}O_5$ requires C, 63·8; H, 7·8 per cent).

Triethyl 3-Methylcyclohexane-1-carboxylate-1-α-glutarate (III). —The acid (60 g.), absolute alcohol (150 c.c.), concentrated sulphuric acid (14 c.c.), 4 litres of alcohol vapourised (4 hours) gave 50 g. of the ester, b. p. 184°/5 mm. (Found: C, 64·09; H, 8·82. $C_{19}H_{32}O_6$ requires C, 64·04; H, 8·9 per cent).

Triethyl 2-methylcyclohexane-1-carboxylate-1-α-glutarate was obtained by the alcohol vapour method. The acid (38 g.) in alcohol (100 c.c.), containing sulphuric acid (10 c.c.), gave 20 g. of the ester on passing 3 litres of alcohol during 3-4 hours, b.p. 187°/4 mm. (Found: C, 64·19; H, 8·63. $C_{19}H_{32}O_6$ requires C, 64·04; H, 8·9 per cent).

Diethyl 3-methylcyclohexane-spiro-cyclopentane-2′-one-3′:5′-dicarboxylate (IV).—A mixture of the foregoing ester (20 g.) and molecular sodium (2·3 g.) and dry benzene (50 c.c.) was refluxed for 10 minutes to start the reaction. The heating was discontinued until the vigour of the reaction abated and was then continued for 2 hours. After cooling, the product was treated with cold dilute sulphuric acid and the benzene layer was washed with aqueous sodium carbonate and with water, dried and evaporated. The residue in alcoholic solution gave a violet colouration with ferric chloride. The ester was obtained as a viscous oil (9 g.), b.p. 185-90°/5 mm. (Found: C, 65·92; H, 8·15. $C_{17}H_{26}O_5$ requires C, 65·8; H, 8·3 per cent).

Diethyl 2-methylcyclohexane-spiro-cyclopentane-2′-one-3′ : 5′-dicarboxylate (IV*a*) was obtained by the above method. In a typical experiment the ester (12 g.) was subjected to the action of molecular sodium (1·4 g.) in dry benzene (40 c.c.), b.p. 180-185°/4 mm., yield 5 g. (Found: C, 66·2; H, 8·4. $C_{17}H_{26}O_5$ requires C, 65·8; H, 8·3 per cent). It gave deep violet colouration with ferric chloride.

3-Methylcyclohexane-spiro-cyclopentane-2′-one-5′-carboxylic Acid (V).—The ester was refluxed with excess of dilute sulphuric acid (20%) for 12 hours and the cooled solution saturated with ammonium sulphate and repeatedly extracted with ether, the extract washed with water and dried with anhydrous sodium sulphate. After removing the ether it was kept in a desiccator where it solidified into a crystalline mass, m.p. 140-43° (after previous softening). (Found: C, 68·69; H, 8·31; Equiv., 209. $C_{12}H_{18}O_3$ requires C, 68·5; H, 8·5 per cent. Equiv., 210).

3-Methylcyclohexane-spiro-cyclopentane-2-one-5′-carboxylic Acid (V).—This acid was also formed when the sodium salt of 1-carboxy-3-methyl-

*cyclo*hexane-1-a-glutaric acid was heated with acetic anhydride. The dry sodium salt (9 g.) and acetic anhydride (10 c.c.) were mixed in a 100 c.c. flask fitted with a condenser. The flask was gradually heated in an oil-bath which was maintained at 130-140° for about 8 hours when the evolution of carbon dioxide was almost over. After removing most of the acetic anhydride uuder reduced pressure, absolute ethyl alcohol (50 c.c.) and concentrated sulphuric acid (10 c.c.) were added, and the mixture heated for 8 hours. After dilution with water, the separated oil was extracted with ether, washed with sodium bicarbonate, dried and the ether removed. It was then heated on a water-bath with aqueous alcoholic caustic soda. After dilution, alcohol was removed and the solution acidified. The gummy mass, which separated, was extracted with ether. After removing ether, the product was kept in a vacuum desiccator, when it solidified, m.p. 140-43° (with previous softening). (Found: C, 68·7; H, 8·4. $C_{12}H_{18}O_3$ requires C, 68·5; H, 8·5 per cent).

Ethyl 3-*methyl*cyclo*hexane-spiro*-cyclo*pentane-2'-one-5'-carboxylate*, prepared by refluxing a solution of the keto-acid (5 g.) in absolute alcohol (20 c.c.) with the addition of absolute alcohol (5 c.c.), saturated at 0° with hydrogen chloride, formed a colourless viscous oil, b. p. 135-140°/4 mm.

The *semicarbazone* crystallised from absolute alcohol, m.p. 185° after previous softening. (Found: N, 13·8. $C_{15}H_{25}O_3N_3$ requires N, 14·2 per cent).

Ethyl 2-*Methyl*cyclo*hexane-spiro*-cyclo*pentane-2'-one-5'-carboxylate*.—The ester (IV) was refluxed with excess of dilute sulphuric acid (20%) for 12 hours and the cooled solution saturated with ammonium sulphate and extracted with ether. On removing ether the keto-acid was obtained as a gum. It was further hydrolysed with alcoholic caustic soda and after working up in the usual manner, was esterified by refluxing (6 hours) a solution of this keto-acid in absolute alcohol, saturated at 0° with hydrogen chloride, b.p. 142-148°/6 mm. (Found: C, 70·7; H, 9·2. $C_{14}H_{22}O_3$ requires C, 70·5; H, 9·2 per cent).

The *semicarbazone* crystallised from ethyl alcohol, m.p. 202°. (Found: N, 14·45. $C_{15}H_{25}O_3N_3$ requires N, 14·24 per cent).

3-*Methyl*cyclo*hexane-spiro*-cyclo*pentane-5'-carboxylic Acid* (VI).—The pure keto-acid (V) (7 g.), amalgamated zinc (45 g.) and concentrated hydrochloric acid (60 c.c.) were refluxed for 12 hours, further quantity of the acid (30 c.c.) was then added and the heating continued for 12 hours. The solution was extracted with ether, and the extract dried with anhydrous

sodium sulphate. After removing the solvent it was kept in a vacuum desiccator when it solidified. It was crystallised from ether, m.p. 62-65° (with previous softening). (Found : C, 73·0 ; H, 9·93. $C_{12}H_{20}O_2$ requires C, 73·4 ; H, 10·2 per cent).

β-Methylnaphthalene. — 3-Methyl*cyclo*hexane-spiro-*cyclo*pentane-5′-carboxylic acid (7 g.) and selenium (25 g.) were heated at 280-90° for 20 hours. and then at 330° for 30 hours. The reaction product was extracted with ether. After removing ether, the residue was distilled over sodium in vacuum. A small quantity of a liquid was collected and converted into picrate, m.p. 115° (mixed m.p. with a genniue sample of the picrate of 3-methyl-naphthalene).

Our respectful thanks are due to Sir P. C. Rây and Dr. P. C. Mitter for their kind interest in the work.

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CHEMICAL EXAMINATION OF *BRAGANTIA WALLICHII* (LOUR.).

By B. L. Manjunath and M. S. Shankara Rao.

The fatty oil from the roots of *Bragantia Wallachii* (Lour.) contains palmitic, lignoceric, oleic and linolic acids.

The yellow substance which separated from the different extracts was identical with *iso*aristolochic acid isolated from *Aristolochia indica*. The isolation of *iso*aristolochic acid from both *Aristolochia indica* and *Bragantia wallichii* is of some phytochemical significance as both the plants belong to the same family.

Bragantia wallichii, Lour. (in Sanskrit Chakrani) belongs to the family of *Aristolochiaceae* and grows on the Western Ghats. The roots in the form of a paste are used in cases of cholera and diarrhœa. Mr. Parthanarayan Pundit of Shimoga informed the authors that about 4 g. of the roots made into a paste with 14 c.c. of lemon juice given orally every fifteen minutes till the acute symptoms disappeared had very beneficial effects. A reference to literature showed that no work had been done on these roots and the roots used in this investigation were collected in Shimoga district, Mysore State.

Experimental.

In order to ascertain the general character of the constituents of the roots, 50 g. of the finely powdered roots were extracted successively with the following solvents in a Soxhlet and the residues from the extracts were dried at 100°.

Solvent.	Percentage of extract.
Petroleum ether (B.p. 50-60°)	0·48%
Ether	0·78
Chloroform	0·49
Ethyl acetate	0·48
Absolute alcohol	3·70

The constituents volatile in steam amounted to 0·05% and an assay showed that the alkaloidal content was 0·03%.

For detailed examination about 50 kg. of the roots were crushed in a disintegrator and were extracted exhaustively with hot alcohol in a big extraction apparatus. The solvent was distilled off and thec oncentrated extract was subjected to steam distillation. The resulting semi-solid residue was separated from the aqueous layer by decanting off the latter.

The aqueous layer was then treated with lead acetate. There was a small amount of precipitate. It was filtered and the filtrate treated with basic lead acetate. The small amount of precipitate which separated was removed by filtration and the filtrate was freed from lead by passing in hydrogen sulphide. The filtrate obtained after removing the lead sulphide was treated with ammonia and extracted with chloroform. The aqueous solution was then concentrated to a small bulk. No solid separated from this on standing and it could not be crystallised from absolute alcohol. It consisted mainly of reducing sugars.

The chloroform layer was extracted with dilute hydrochloric acid. This (A) was later worked with the acid extracts of ether and chloroform extracts described later.

The semi-solid residue left after steam distillation was dissolved in sufficient alcohol and the alcoholic solution was dried over 1 kg. of the extracted roots. It was then thoroughly extracted in a small copper Soxhlet with solvents in the order mentioned above.

The Petroleum Ether Extract.—This extract consisted of a dark brown oil (450 g.).

The oil was examined according to the usual methods of analysis. The solid acids consisted of palmitic acid (m.p. 62° ; M.W., 256 ; m.p. of *p*-phenylphenacyl derivative, 93·5°) and a small amount of crude lignoceric acid (m.p. 75° ; M.W., 370 ; m.p. of *p*-phenylphenacyl derivative, 101°). The liquid acids were found to consist of oleic acid (dyhydroxystearic acid, m.p. 131° ; M.W., 315) and linolic acid [tetrahydroxystearic acid m.p. 163° ; M.W., 352 ; tetrabromostearic acid, m.p. 114° ; M.W., 595]. The unsaponifiable matter consisted of a thick brown resinous material and glycerol was identified among the products of saponification.

The Ether Extract.—As the extraction proceeded a yellow substance separated out from the solvent. The ether solution was successively extracted with 5% hydrochloric acid, 5% potassium bicarbonate, 5% potassium carbonate and 5% potassium hydroxide. The hydrochloric acid extract was worked later with the hydrochloric acid extract of the chloroform extract.

The same yellow substance also separated out on acidifying the bicarbonate and the carbonate extracts. No pure substance could be isolated from the alkali extract and from the neutral residue in ether.

The Chloroform Extract.—The chloroform solution was extracted thoroughly with 1% hydrochloric acid. This extract was combined with the hydrochloric acid extracts of the ether extract and the acid extract (A). The acid extract gave positive tests with alkaloidal

reagents. On the addition of excess of dilute ammonia a brownish slimy precipitate was obtained and it was extracted with chloroform. The basic material was then taken up by hydrochloric acid and re-extracted with chloroform and the solution dried over anhydrous sodium sulphate. When the solvent was evaporated off under reduced pressure the residue (0·5 g.) was very easily oxidised on exposure to air and all attempts to obtain it in a pure condition proved fruitless.

The original chloroform extract was then extracted successively with 5 % potassium bicarbonate, 5% potassium carbonate and 5% potassium hydroxide. Some more of the yellow material separated on acidifying the bicarbonate and the carbonate extracts, but no pure compound could be obtained from the alkali extract and the neutral residue.

The Ethyl Acetate Extract.—The only pure material that could be isolated from this extract also consisted of the yellow material already mentioned.

The Alcoholic Extract.—The final alcoholic extract did not yield any definite substance.

Isolation of isoAristolochic Acid.

The yellow material from the ether, chloroform and ethyl acetate extracts was a single compound. It was first crystallised from glacial acetic acid and then from 96% alcohol (charcoal) when it was obtained in the form of yellow rectangular plates, m.p. 278° (decomp.). The substance did not depress the m.p. of pure *iso*aristolochic acid (Manjunath, Venkata Rao and Krishnaswamy, *J. Indian Chem. Soc.*, 1935, **12**, 482). It was intensely bitter and dissolved in alkali forming a red solution. The composition of the substance corresponded to the formula $C_{17}H_{11}O_7N$. (Found : C, 60·08 ; H, 3·6 ; N, 4·26 ; M.W. in nitrobenzene by boiling point method, 351·6. $C_{17}H_{11}O_7N$ requires C, 59·8 ; H, 3·3 ; N, 4·11 per cent. M.W., 341·0).

The methyl derivative of the substance was prepared by heating it with methyl sulphate and alkali. It crystallised from ethyl benzoate in yellow silky needles (m.p. 269°) and did not depress the m.p. of the methyl derivative of *iso*aristolochic acid. Unlike the acid the methyl derivative was quite tasteless.

The work described above formed part of the thesis submitted by one of us (M.S.S.) for the degree of Master of Science in the Mysore University. We are grateful to the University for permission to publish the same.

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INTERACTION OF SULPHURYL CHLORIDE WITH ARYLAMIDES OF AROMATIC ACIDS. PART I.

By G. V. Jadhav and D. R. Sukhtankar.

Sulphuryl chloride gives chlorinated products with arylamides of benzoic acid. Chlorine first enters the basic part of the molecule in position *para* to the amido group and with excess of sulphuryl chloride dichloro derivatives are formed, the second chlorine atom entering the same nucleus. The constitution of the compounds is proved by hydrolysis as well as by synthesis.

Sulphuryl chloride has been used as a chlorinating agent in the case of aromatic compounds with various substituents like OR, NR_2, COOH, etc. and it has been noted that its reactivity depends upon the substituents in the nucleus.

Although aromatic hydrocarbons give chloro derivatives, introduction of an anionoid group like NH_2, greately increases the reactivity of the molecule (*cf.* Wenghöffer, *J. pr. Chem.*, 1877, *ii*, **16**, 449). When, however, the basic character of the amine is neutralised by acylation, either a dichloro or a monochloro derivative is obtained as is shown by Wenghöffer (*loc. cit.*) and Wynne (*J. Chem. Soc.*, 1892, **61**, 1042).

Benzarylamides are very reactive towards sulphuryl chloride and the reaction products cannot be isolated. Use of dry benzene is found convenient to control the vigour of the reaction. Action of sulphuryl chloride has been attempted on benzoyl derivatives of aniline, *o*-and *m*-chloroanilines, *o*-, *m*- and *p*-toluidines, *p*-anisidine, *p*-phenetidine, α- and β-naphthylamines, *o*-, *m*- and *p*- nitroanilines and *o*-nitro-*p*-toluidine.

Benz-nitroarylamides, except benz-*o*-nitroanilide, failed to give reaction products due to the deactivating influence of the nitro group in the molecule.

The constitution of halogenated products is proved by (*i*) hydrolysis and identifying the chloroamine obtained or its acetyl derivative as well as the acid, by mixed melting point with an authentic specimen and (*ii*) by mixed melting point of the reaction product with benzoyl derivative of the chloroamine.

It has been observed that chlorine generally first enters the position *para* to the amido group whenever free and when excess of sulphuryl chloride is used, to *ortho* as is found in the case of anilide, *m*-toluidide, *o*-toluidide and α-naphthylamide.

It seems that the orienting influences of methoxy and amido groups are nearly equal because dichloro derivative of benz-*p*-anisidide can always be obtained under experimental conditions.

Although it is well known that *o*-*p*- directing influence of the amido group is more powerful than alkoxy group, benz-2-chloro-*p*-phenetidide ($OC_2H_5 = 1$) can be isolated, together with benz-2:5-dichloro-*p*-phenetidide in the chlorination of benz-*p*-phenetidide with sulphuryl chloride.

In the case of benz-*m*-toluidide, excess of sulphuryl chloride gave two chloro derivatives, one of which (m.p. 111-12°) has been proved to be benz-4:6-dichloro-*m*-toluidide (Me=1) both by hydrolysis to 4:6-dichloro-3-aminotoluene and by preparing it from 4:6-dichloro-3-aminotoluene by benzoylation. The other compound (m.p. 147-48°) is considered to be benz-2:6-dichloro-*m*-toluidide (Me=1) because the melting point of the corresponding acetyl derivative (m.p. 117°) agrees well with that described by Cohen and Dakin (*J. Chem. Soc.*, 1902, **81**, 1331). Only other constitution possible for that compound is benz-5:6-dichloro-*m*-toluidide, but the melting point of the acetyl derivative of 5:6-dichloro-*m*-toluidine is 187°.

EXPERIMENTAL.

Sulphuryl chloride was gradually added to the amide suspended in dry benzene (about 40 c.c. for 5 g. of amide) and the mixture was allowed to stand overnight at room temperature being protected from moisture. As the reaction started, the amide dissolved in the solvent and the solution was then boiled under reflux for about 4 hours, except in the case of benz-*p*-anisidide (1:1) when it was boiled for 2 hours only. The reaction product, after removal of benzene, was washed free from sulphuryl chloride with dry petroleum. The substances were generally soluble in benzene, alcohol and acetic acid and were crystallised from alcohol in colourless needles, except in the case of benz-*o*-nitroanilide when it was yellowish.

In the case of benz-*p*-toluidide and benz-*p*-phenetidide, where two products are simultaneously formed, they were separated by fractional crystallisation from alcohol.

Constitution of products marked (A) is proved by hydrolysis to the known amine and benzoylation of the corresponding amine.

Substances marked (B) are identified by hydrolysis to the corresponding amine only and those marked (C) by synthesis only. In the case of substances marked (D) in addition to (A) or (B), the amine was identified through its acetyl derivative (by mixed melting point with an authentic sample). Benz-2-nitro-4-chloroanilide was prepared by nitrating benz-*p*-chloroanilide with dilute nitric acid (1:1). In the case of chloro derivatives of benzanilide, benz-*o*-toluidide, benz-*m*-chloroanilide and monochloro derivative of benz-*m*-toluidide, hydrolysis was carried out by boiling the substances with 50% alcoholic caustic patash solution for about 6 hours. Remaining substances were hydrolysed by heating with 50% alcoholic caustic potash solution in a sealed tube at 160-70° for about 6 hours.

The following table shows the details of the substances.

TABLE I

Amide.	Proportion of amide to SO_2Cl_2.	Product.	M.p.	Formula.	Analysis Found.	Analysis Required.	Yield.
Benzanilide	1 :1	Benz-*p*-chloroanilide (A&D)	190-91°	$C_{13}H_{10}ONCl$	Cl, 15·3%	Cl, 15·3%	90%
Benzanilide	1 :2	Benz-2 :4-dichloro-anilide (A)	115-16°	$C_{13}H_9ONCl_2$	Cl, 26·6	Cl, 26·7	80
Benz-*o*-chloroanilide	1 :1	Benz-2 :4-dichloro-anilide*	115-16°	$C_{13}H_9ONCl_2$	Cl, 26·5	Cl, 26·7	80
Benz-*m*-chloroanilide	1 :1	Benz-3 :4-dichloro-anilide (A)	143-44°	$C_{13}H_9ONCl_2$	Cl, 26·5	Cl, 26·7	75
Benz-*o*-toluidide	1 :1	Benz-5-chloro-*o*-toluidide (A&D)	162-63°	$C_{14}H_{12}ONCl$	Cl, 14·4	Cl, 14·5	90
Benz-*o*-toluidide	1 :2	Benz-5-chloro-*o*-toluidide*	162-63°	$C_{14}H_{12}ONCl$	—	—	—
Benz-*m*-toluidide	1 :1	Benz-6-chloro-*m*-toluidide (A)	116-18°	$C_{14}H_{12}ONCl$	Cl, 14·3	Cl, 14·5	—
Benz-*m*-toluidide	1 :2	(*i*) Benz-2 :6-dichloro-*m*-toluidide (B&D)	147-48°	$C_{14}H_{11}ONCl_2$	Cl, 25·1	Cl, 25·3	20
		(*ii*) Benz-4 :6-dichloro-*m*-toluidide (A)	111-12°	$C_{14}H_{11}ONCl_2$	Cl, 25·2 N, 5·1	Cl, 25·3 N, 5·0	70
Benz-*p*-toluidide	1 :1	Benz-3 chloro-*p*-toluidide (A&D)	139°	$C_{14}H_{12}ONCl$	Cl, 14·2	Cl, 14·5	—
Benz *p*-toluidide	1 :2	(*i*) Benz-3 :5-dichloro-*p*-toluidide (A&D)	114-115°	$C_{14}H_{11}ONCl_2$	Cl, 25·2	Cl, 25·3	—
		(*ii*) Benz-3-chloro-*p*-toluidide*	138°	$C_{14}H_{12}ONCl$			

TABLE I (*contd.*).

Amide.	Proportion of amide to SO_2Cl_2.	Product.	M.p.	Formula.	Analysis Found.	Analysis Required.	Yield.
Benz-*p*-anisidide	1 :1	Benz-2 :5-dichloro-*p*-anisidide *	136-37°	$C_{14}H_{11}O_2NCl_2$	Cl, 23·7%	Cl, 23·9%	—
Benz-*p*-anisidide	1 :2	Benz-2 :5-dichloro-*p*-anisidide (C)	136-37°	$C_{14}H_{11}O_2NCl_2$	Cl, 23·7	Cl, 23·9	70%
Benz-*p*-phenetidide	1 :1	(*i*) Benz-2-chloro-*p*-phenetidide (C)	171-72°	$C_{15}H_{14}O_2NCl$	Cl, 12·7	Cl, 12·9	20
		(*ii*) Benz-2 :5-dichloro-*p*-phenetidide (C)	143-44°	$C_{15}H_{13}O_2NCl_2$	Cl, 22·7	Cl, 22·9	60
Benz-*p*-phenetidide	1 :2	Benz-2 :5-dichloro-*p*-phenetidide*	144-45°	—	—	—	—
Benz-α naphthylamide	1 :1	Benz-4-chloro-α-naphthylamide (A)	226°	$C_{17}H_{12}ONCl$	Cl, 12·7	Cl, 12·7	85
Benz-α-naphthylamide	1 :2	Benz-2 :4-dichloro-α-naphthylamide (A)	212-213°	$C_{17}H_{11}ONCl_2$	Cl, 22·3	Cl, 22·5	70
Benz-β-naphthylamide	1 :1	Benz-1-chloro-β-naphthylamide (A)	171°	$C_{17}H_{12}ONCl$	Cl, 12·6	Cl, 12·7	80
Benz-*o*-nitroanilide	1 :1	Benz-2-nitro-4-chloroanilide (D)	130°	$C_{13}H_9O_3N_2Cl$	Cl, 12·6 N, 10·1	Cl, 12·8 N, 10·2	

N.B.—(*i*) In the case of toluidides, anisidide and phenetidide Me, OMe, and OEt are numbered 1.
(*ii*) Substances marked* were identified by mixed m.p. with authentic specimens.

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A NOTE ON THE OCCURRENCE OF BEHENIC ACID IN THE OIL FROM THE SEEDS OF *PONGAMIA GLABRA*, VENT.*

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The refined oil from the seeds of *Pongamia Glabra* (Hongey in Kannada, Karanja and Naktamala in Sanskrit and Sukchain in Hindi) has been subjected to detailed analysis by Sudborough, Watson, and Desai (*J. Indian Inst. Sci.*, 1923, **6**, 93) and it has been found to contain the following fatty acids:

(*i*) Myristic acid, (*ii*) palmitic acid, (*iii*) stearic acid, (*iv*) arachidic acid, (*v*) lignoceric acid, (*vi*) dihydroxystearic acid, (*vii*) linolic acid, (*viii*) linolenic acid and (*ix*) oleic acid.

While examining the solid which gradually separates out on allowing the crude oil to stand for several days, evidence was obtained for the presence of behenic acid also in the oil.

The solid residue which separates from the oil was repeatedly washed with methyl alcohol to remove the resinous matter. The residue was then extracted with hot ethyl alcohol in a Soxhlet. After all the karanjin (Beal and Katti, *Zentrl.*, 1926, **II**, 596; Limaye, *Proc. Indian Sci. Congress*, 1925, p. 118) was extracted, there remained a residue which was very sparingly soluble in boiling alcohol and was found to be a mixture of zinc salts of fatty acids.

From this the acids were liberated and their methyl esters were distilled under reduced pressure (0·5 mm.), when two principal fractions were obtained.

The free acid obtained from the first of these (b. p. 199-205°/0·5 mm.) on repeated crystallisation from methyl alcohol melted at 78-79°. (Found: M. W., 342·3. $C_{22}H_{44}O_2$ requires M. W., 340). It was, therefore, identified as behenic acid.

The free acid from the second fraction (b. p. 207-212°/0·5 mm.) and residues in the distillation flask on repeated crystallisation from methyl alcohol melted a 74° (Found: M. W., 371·3. $C_{24}H_{48}O_2$ requires M. W., 368·0) and did not depress the m. p. of crude lignoceric acid (m. p. 74°) from natural sources.

Since Sudborough *et al* (*loc. cit.*) have not found behenic acid in the purified oil it is likely that this acid occurs free in the oil and is removed during the process of purification.

* The work formed part of the thesis submitted by one of us (M. S. S.) for the degree of Master of Science in the Mysore University. We are indebted to the University for permission to publish the same.

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